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PHYSIOLOGICAL REVIEWS

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PHYSIOLOGICAL REVIEWS

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PHYSIOLOGICAL REVIEWS

VOL. 24 OCTOBER, 1944 No. 4

FACTORS AFFECTING THE INSULIN CONTENT OF THE PANCREAS

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The pancreas as the source of insulin. Insulin, the anti-diabetic hormone, is produced in the pancreas (1) and, despite the earlier literature to the contrary, there is no good evidence that it is produced in any other organ or tissue (2). The beta cells of the islets of Langerhans are conceded to be the secreting structures. The demonstration that insulin can be obtained from the principal islets of teleost fishes and not from acinous tissue (3) and that it is found in metastatic islet cell tumors (4) indicates that the islets of Langerhans are responsible for the production of this material. That the beta cells are the actual secreting elements seems likely from the fact that the severity of the diabetes in partially depancreatized dogs (5) (6) or in dogs injected with anterior pituitary extract (7) (8) (9) parallels the extent of the change in the beta cells of the islets. Indeed, the degree of beta cell granulation demonstrated with Bowie's staining technique in the islets of pituitary-injected dogs (7) (8) (9) (10) and in the islets of fasting and fat-fed rats given insulin (11) shows a close correlation with the insulin content of the pancreas.

The significance of the insulin level. If it is granted that the beta cells of the islets of Langerhans in the pancreas are the insulin-producing cells, then it will be apparent that the insulin content of the pancreas is dependent upon *1*, the *concentration* of insulin in the beta cells of the islets, and *2*, the total volume of the beta cells. It follows then that some of the factors influencing the insulin content of the pancreas will bring about their effects by altering islet volume, whereas others will change the concentration of insulin in the islet cells. If the beta cell volume remains constant, at any moment the concentration of insulin in the pancreas will represent a balance between the production and liberation of that material. Such a balance might be struck at any level, so some level-regulating mechanism must be postulated. Many of the same difficulties are involved in a discussion of the level of pancreatic insulin as in a consideration of the blood sugar level (12) or, for that matter, the level of any material in the body normally present in fairly constant amounts.

At any level, the turnover of insulin might conceivably be great or small but so long as production and liberation keep pace the level will remain constant. The fact that when only a fraction of the pancreas remains, the animal may be free from diabetes and that under these circumstances the pancreatic insulin concentration may be unchanged (13) (14) would indicate either that only a fraction of the pancreas is functioning under normal conditions or that there is a greater than normal turnover of insulin in the remnant without any great change in level. Nothing is known of the mechanism which stimulates or diminishes production when the liberation of insulin is altered, yet some such regulation under a fairly wide range of conditions would seem to be a necessity.

It should be emphasized, however, that the insulin level as measured by any of the present methods is sufficiently variable to make measurements of small changes in content uncertain and to make conclusions on the basis of such changes unwarranted. Such small changes may be significantly concerned with the normal variation in production and liberation. Studies on the partially depancreatized non-diabetic dog (13) (14) would indicate that the concentration or level of insulin in the beta cells is probably of more significance than the total content, since in these animals the total content may be drastically reduced (by operation) without greatly changing the level of insulin in the pancreas or altering the state of the animal.

A lowering of the insulin content of the pancreas must result from either a reduction in the number of functioning beta cells or some disparity between the production and liberation of insulin. The disparity may be caused by a reduction in the manufacture of insulin that is out of proportion to any change in liberation, or an increased liberation of insulin that is out of proportion to any change in production. A reduction in the insulin content of the pancreas may result then from an increased liberation of insulin, a decreased production of insulin or both. Once the new level is reached, it may be maintained by again balancing production and liberation. What regulates turnover at any given level or operates to alter the level is the unknown but important factor. It is obvious that a determination of the level of pancreatic insulin *per se* may tell little of what is happening in the pancreas. Many determinations must be made under a variety of experimental conditions and correlated with associated metabolic and histological changes if the significance of the insulin level is to be understood.

Methods involved in the determination of the insulin content of pancreas. The estimation of the insulin content of the pancreas under most circumstances involves the removal of all the pancreas from the animal, the extraction of the tissue removed and the determination of the insulin content of the resultant solution. The removal of the pancreas must be complete, and all the pancreatic tissue should be extracted. This is particularly important in those animals having a pancreas that is diffusely distributed and may contain varying amounts of fat. Also, since the concentration of insulin is not the same in all parts of the pancreas (14) a sample portion may give an erroneous impression of the insulin content.

The extraction procedures have differed considerably. A great deal of work has been done on this problem and its discussion is beyond the scope of this paper. Perhaps the most commonly used extraction fluid is acid alcohol. Much of the recent work employs Scott and Fisher's modification (16) of the procedure of Jephcott (15). It should be pointed out that in using this procedure the recovery of crude insulin added to the tissue is good, while the recovery of purified insulin is poor and variable (0–96 per cent) (15). Marks and Young (17) commented on their inability to recover all added insulin by the method of Scott and Fisher, but failed to note Jephcott's earlier observations. Since crude insulin is recovered almost completely, the method would seem to be more satisfactory than the partial recovery of added purified insulin would indicate. In tests made on

aliquots of two homogeneous preparations of pancreatic tissue obtained in each instance from 50 rats, Best, Haist and Ridout (23) (73) found this extraction procedure to give remarkably consistent results.

The methods of measuring the insulin potency of the pancreatic extracts differ considerably in the different laboratories. Both rabbit and mouse assay procedures have been employed but the latter have been used more commonly for the experimental investigations. The "mouse" methods depend upon injecting the test solution into mice and noting the number of mice which convulse. This is compared with the effect of a standard insulin solution. Some workers use several dilutions of unknown and standard solutions and compare the relation of the two convulsion-dose curves (18) (19). Others use one dilution of standard and one of unknown that causes approximately 50 per cent of the mice to convulse. A large number of mice are tested at this dilution and the relation of the potencies of unknown and standard are found by reference to a characteristic dose-response curve previously obtained on a large number of mice (20) (21). A comparison of the methods is given by Gaddum (22). The accuracy of either procedure is dependent upon the number of mice used. The number used constitutes an important point of difference between the tests as carried out by different workers and is not often mentioned in their reports.

Manner of expressing results. The concentration of insulin in the beta cells of the islets is undoubtedly the important value to determine but since the simultaneous measurement of beta cell volume and insulin content of pancreas is not feasible, other methods of expressing the results must be followed. The way in which the results are expressed differs in the various reports. Some give the results as units of insulin per kilogram of body weight, others as units per gram or kilogram of pancreas and others as units per rat or group of rats. None of the methods of presenting the results is satisfactory under all circumstances. In those animals having a diffusely distributed pancreas, any expression on the basis of weight of pancreatic tissue becomes of doubtful value because variable amounts of fat and other material usually are present (23). In the rat, whose pancreas is diffuse, it is convenient to compare the total insulin content of the pancreas of the rats or groups of rats. This method is of value only if the animals in control and test groups are as nearly similar as possible at the start of the experiment. When a relatively homogeneous population of cases can be obtained, it is the method of choice. Expressions on the basis of body weight are useful, though here too it is possible to fall into error. Frequently there is no comment to indicate whether the reference is to the initial or final weight of the test animals. The comparison of insulin contents based on the final body weight is sometimes misleading. For example if, in the test group, the loss in body weight is greater than the reduction in insulin content and the results are expressed in terms of final body weight, then an increase over the control group will be indicated whereas no actual increase has occurred and the total content may be even less than in the controls. Under varying conditions the insulin content of the pancreas need not bear any specific relationship to body weight (23), though it is true that in *normal untreated* rats

there is a rough relationship between insulin content and body weight (24) (25). In dogs, the population of cases is not homogeneous and here it is frequently more illuminating to indicate the *concentration* of insulin as well as the total amount of insulin in the pancreas.

TABLE 1

Insulin content of pancreas in different species

SOURCE	UNITS/GRAM PANCREAS	WORKER	METHOD	DATE
Birds				
Chicken	0.76	Redenbaugh, Ivy and Koppanyi (31)	Alcohol-H_2SO_4	1926
Chicken	0.65	Jephcott (32)	Alcohol-HCl	1931
Duck	0.53	Jephcott (32)	Alcohol-HCl	1931
Mammals				
Mouse	1.7	Marks and Young (26)		1940
Rat (Wistar)	2.1	Best, Haist and Ridout (23)	Alcohol-HCl	1939
Rat (Wistar)	0.6	Soong (33)	Alcohol-HCl	1940
Rat (Wistar)	1.3	Marks and Young (26)	Alcohol-HCl	1940
Rat (Wistar)	1.0	Griffiths (24)	Alcohol-HCl	1941
Rabbit	7.8	Marks and Young (26)	Picrate-acetone	1940
Rabbit	9.5	Marks and Young (17)	Alcohol-HCl	1940
Rabbit	5.2	Griffiths (34)	Alcohol-HCl	1942
Cat	2.0	Baker, Dickens and Dodds (35)	Acetone-picric acid	1924
Cat	0.68	Takeuchi (36)	Acetone-picric acid	1928
Cat	2.5	Jephcott (32)	Alcohol-HCl	1931
Cat	1.7	Scott and Fisher (16)	Alcohol-HCl	1938
Cat	2.2	Marks and Young (26)	Alcohol-HCl	1940
Dog	0.8 -1.5	Nothmann (37)	Alcohol-H_2SO_4	1925
Dog	2.1	Takeuchi (36)	Alcohol-H_2SO_4	1928
Dog	2.6	Takeuchi (36)	Acetone-picric acid	1928
Dog	3.8	Jephcott (32)	Alcohol-HCl	1931
Dog	5.0 -5.5	Murray and Waters (38)	Alcohol-HCl	1932
Dog	3.4	Best, Campbell and Haist (7)	Alcohol-HCl	1939
Dog	3.3	Marks and Young (26)	Alcohol-HCl	1940
Guinea pig	0.08	Marks and Young (26)	Alcohol-HCl	1940
	0.23	Marks and Young (26)	Picrate-acetone	
Beef	0.75-1.2	Wernicke (39)	Alcohol-HCl	1924
Beef	1.5 -2.5	Somogyi, Doisy and Shaffer (40)	Alcohol-H_2SO_4	1924
Beef	0.9 -1.5	Dudley and Starling (41)	Alcohol-$NaHCO_3$	1924

TABLE 1—*Concluded*

SOURCE	UNITS/GRAM PANCREAS	WORKER	METHOD	DATE
Mammals—Cont'd				
Beef	2.5	Baker, Dickens and Dodds (35)	Acetone-picric acid	1924
Beef	4.4	Moloney and Findley (42)	Alcohol	1924
Beef	1.8	Fenger and Wilson (43)	Alcohol-HCl	1924
Beef	2.0	Langecker and Wiechowski (44)	Acid-alcohol	1925
Beef	1.8 -2.2	Scott and Best (45)	Alcohol-H_2SO_4	1925
Beef	3.3	Scott (46)	Alcohol-HCl	1925
Beef	1.8 -2.5	Blatherwick et al. (47)	Alcohol-H_2SO_4	1927
Beef	1.3	Takeuchi (36)	Acetone-picric acid	1928
Beef	1.3	Kaulbersz (48)	Alcohol-H_2SO_4	1930
Beef	1.4	Kaulbersz (48)	Alcohol-$NaHCO_3$	
Beef	1.4	Kaulbersz (48)	Water-HCl	
Beef	1.5		Water-$NaHCO_3$	
Beef	3.0 -3.5	Jephcott (15)	Alcohol-HCl	1931
Beef (2 years)	4.8	Fisher and Scott (49)	Alcohol-HCl	1934
Beef (9 years and older)	1.8	Fisher and Scott (49)	Alcohol-HCl	1934
Pig	1.6 -3.1	Dodds and Dickens (50)	Water, formic acid and formaldehyde	1924
Pig	5.5	Dodds and Dickens (50)	Acetone-picric acid	1924
Pig	3.0	Clough, Allen and Murlin (51)	Water-HCl	1924
Pig	3.0 -4.3	Baker, Dickens and Dodds (35)	Acetone-picric acid	1924
Pig	1.7 -1.9	Fenger and Wilson (43)	Alcohol-HCl	1924
Pig	2.1	Takeuchi (36)	Alcohol-H_2SO_4	1928
Pig	1.3	Takeuchi (36)	Acetone-picric acid	1928
Pig	2.0	Jephcott (32)	Alcohol-HCl	1931
Sheep	1.0	Baker, Dickens and Dodds (35)	Acetone-picric acid	1924
Sheep	1.8	Fenger and Wilson (43)	Alcohol-HCl	1924
Sheep	0.7	Jephcott (32)	Alcohol-HCl	1931
Horse	1.5	Baker, Dickens and Dodds (35)	Acetone-picric acid	1924
Horse	1.4	Takeuchi (36)	Acetone-picric acid	1928
Horse	2.0	Jephcott (32)	Alcohol-HCl	1931
Monkey (Macacus rhesus)	2.5	Jephcott (32)	Alcohol-HCl	1931
Chimpanzee	11.2	Marks and Young (26)	Alcohol-HCl	1940
Man	0.24	Pollak (52)		1926
Man	0.84	Takeuchi (36)		1928
Man	1.7	Scott and Fisher (53)	Alcohol-HCl	1938

Insulin content in normal animals of different species. The total insulin content of the pancreas obviously will vary greatly from species to species. The concentration of insulin in the gland, however, is far more constant. Normal values have been reported for the following: fish, chicken, duck, mouse, rat, guinea pig, rabbit, cat, dog, cattle, pig, sheep, horse, monkey, chimpanzee and man. The values obtained, the extraction method used, the references and dates are given in table 1. Some unpublished data obtained by C. M. Jephcott in 1931 are included in the table.

It will be evident that the concentration of insulin in the pancreas of mammals is somewhat similar in the different species. The values for the rabbit and chimpanzee are high, while those for the guinea pig are unexpectedly low (26). Judging from the duck and chicken, fowl have lower concentrations of insulin in their pancreases than most mammals. The values in teleost fish, which are not included in the table but range from 2 to 13 units per gram, can hardly be compared with the others since only the principal islets were used for extraction and these are composed almost entirely of islet tissue (27) (28) (29). Using the $\frac{\text{islet} \times 100}{\text{acinar}}$ ratio of 1.1 obtained by Richardson and Young (30) for Wistar rats, and insulin values per gram of pancreas obtained by Marks and Young (17) and by Best, Haist and Ridout (23), it can be calculated that the insulin per gram of *islet* tissue for the Wistar rat is somewhere in the neighborhood of 119–193 units, a value much higher than any reported for the principal islets of fish. The values for the insulin content per kilo of body weight of animal, though not given in the table, do not show a great range, indicating that in normal animals the total insulin content is roughly proportional to body weight. Considerable variation in insulin concentration and total content have been reported for single species. One important factor in these differences is the age of the animals.

Insulin content and age. The insulin content of the pancreas changes with age. In the cow, the *concentration* of insulin in the pancreas decreases as the animals become older (49). Fetal calves under 5 months show an average of 33.2 units per gram, fetal calves 5 to 7 months, 23.1 units, calves 6 to 8 weeks old, 11.4 units, 2-year heifers, 4.8 units, cows 9 years and older, 1.8 units per gram of pancreas. The *total insulin content* of the pancreas of Wistar rats, however, increases with age (24) (25). In normal humans above 12 years of age the pancreatic insulin does not seem to be affected by length of life (53). However, data on islet volume changes would indicate that during the first few years after birth the insulin content of human pancreas probably would show a substantial increase with age. Ogilvie (54), in an intensive study of islet and acinar volumes and islet counts in the pancreases of 100 human subjects varying from birth to 64 years of age, found that the average islet weight per pancreas increased from 0.12 gram at birth to 1.07 grams at 21 years. The weight of islet tissue varied greatly, however, in any age group. There was a rapid increase in islet tissue at first, with a tendency to level off at the third year but with a rapid increase again during adolescence, and a final stabilization at about 21

years. The total islet count increased from an average of 284,000 at birth to an average of 960,000 at 3 years, after which it remained relatively constant. In the first few years of life therefore the increase in the number of islets seems to account for the increase in islet weight, whereas during childhood and adolescence the increase appears to be brought about by an increase in the size of the islets. After 21 years of age the amount of islet tissue per kilogram of body weight is fairly constant, though after 45 years of age the increase in body weight is greater than that of islet tissue (54).

While in certain species, then, the *concentration* of insulin in the pancreas is higher in fetuses and very young animals, yet in others it would appear that the *total* insulin content of pancreas increases with age though it may tend to level off when adult life is reached.

Seasonal variation in insulin content. Fisher and Scott (49) found that better yields of insulin were obtained from beef pancreas during the winter months. Nitzescu (55) obtained greater yields from June to September than from November to February. However, other factors, such as age and the freshness of the tissue, which usually are not well controlled in studies on beef pancreas, may be involved in these variations. The insulin content of pancreas does seem to fluctuate when observed over a period of time in carefully controlled experiments but the factors involved are not clear. No seasonal relationship has been definitely established.

Distribution of insulin in the pancreas. The distribution of insulin in the pancreas of the dog is not uniform. It was found that the free splenic end of the pancreas had the highest content of insulin (4.2 units per gram). The attached duodenal portion was next (3.1 units per gram) and the free duodenal end had the lowest concentration (2.2 units per gram) (14). Figures are not available concerning the distribution of insulin in the pancreas of other animals, but the above order of distribution was observed for islet counts in guinea pigs (56). In humans also the greatest number of islets was found in the splenic end of the pancreas (57). The data do not allow a good comparison of the distribution of insulin in the pancreases of different species. There is sufficient evidence, however, to indicate that the concentration of insulin is not uniform throughout the whole pancreas, a fact that must always be kept in mind when a part of the pancreas is taken for extraction.

Insulin content and anesthesia. Anesthesia with urethane and sodium amytal was found by Best, Haist and Ridout (23) to cause no appreciable alteration in the insulin content of pancreas as compared with the values obtained in stunned rats. This was true despite the fact that the blood sugar was greatly elevated in the group receiving urethane. The effect of other anesthetics has not been reported.

Ligation of ducts. Herxheimer (58) showed that extracts of duct-ligated pancreas were more effective in lowering blood sugar than extracts of normal glands. Ligation of the ducts leads to atrophy of the pancreas as a whole, though it has been reported to cause regeneration of the islets (59) (60). Atrophy of acinous tissue occurs more rapidly than that of the islets in duct-ligated

pancreas. The original islets, however, atrophy also and regeneration does not restore the islets to their normal state (61). This differential atrophy leads to an increase in the islet-acinar ratio and would account for an increased insulin *concentration* but a decrease rather than an increase in *total content* would be expected. Such a reduction in the total insulin content of the pancreas has been noted by Waters and Best in dogs 8 to 10 weeks after ligation of the pancreatic ducts (personal communication).

Infection. Menten and Krugh (62) were unable to determine any appreciable alteration in the insulin content of the rabbit pancreas following single intravenous sublethal doses of paratyphoid B. filtrates, despite the fact that hydropic degeneration of islet cells may occur in rabbits and guinea pigs suffering from infection with enteritides-paratyphosus B. group, and despite the fact that injections of paratyphoid B. filtrates produce destruction of the principal islets of certain species of teleost fish. It is well to note that the normal values they report are surprisingly low. Jephcott (personal communication) found an insulin content of 1.4 units per gram pancreas in a monkey dying from massive tuberculosis, whereas values in normal monkeys averaged 2.5 units per gram. Murray and Waters (38) induced infection in dogs by ligating the appendix and wrapping it with omentum. They concluded that their results showed "that there is a significant decrease in the insulin content of the pancreas of dogs suffering from an acute infection associated with fever and accompanied by suppurative processes." It might be pointed out that the normal values they give are high and that the concentrations in the infected animals were within the range of normal values reported by other workers. It is possible that this reduction may simply reflect a diminished food intake, since undernutrition itself has subsequently been shown to lower insulin content (63). Some insulin assays done in our laboratory for T. F. Nicholson showed that the insulin content of pancreas in rats injected with staphylococcus toxin was not significantly different from that of control animals receiving the same caloric intake.

Insulin and tumors. Cori (64) was unable to find more than a trace of insulin-like substance in a variety of benign and malignant tumors. Cramer, Dickens and Dodds (65) found also that a number of different tumors yielded no appreciable amount of insulin.

Ruffo and Correa (66) isolated a substance from spindle-celled sarcomata in rats which when injected lowered the blood sugar level. While the existence of insulin in tumor tissue in general seems unlikely, its presence in certain pancreatic tumors and their metastases is well established. Wilder, Allen, Power and Robertson (4) reported a case with carcinoma of the islets of Langerhans and metastatic tumors in the liver. Tumor nodules taken from the liver were extracted and tested for insulin. They found from $\frac{1}{4}$ to $\frac{1}{2}$ as high an insulin concentration in the metastatic tumor tissue as they normally obtained from pancreas (0.4 clinical unit (0.13 unit) per gram). Power, Cragg and Lindem (67) also reported that insulin was found in metastatic islet cell tumors in the liver. Derick and associates (68) found that a saline extract of an islet cell tumor had an insulin-like effect, and Graham and Womack (69) estimated that

4 units of insulin were present in 1 gram of tumor tissue from one of their patients. Campbell, Graham and Robinson (70), reporting on several cases with islet cell tumors of the pancreas, found that the insulin concentration in the tumors was 4–40 times that of the normal pancreas. A malignant tumor arising from aberrent pancreatic tissue in the liver was shown by Ballinger (71) to contain an insulin-like material.

It has already been pointed out that the calculated concentration of insulin in *islet* tissue for the Wistar rat is 119 to 193 units per gram. Using an $\frac{\text{islet} \times 100}{\text{acinar}}$ ratio of 1.62 for humans at 21 years of age, calculated from Ogilvie's data (54), and insulin concentrations in normal human pancreas obtained by Scott and Fisher (53), the insulin concentration in pure human *islet* tissue is found to be 107 units per gram on the average and as high as 238 units per gram in individual cases. These are only very approximate values but they serve to show that the insulin levels for islet tumor tissue published in most reports are lower than would be expected. However, recently D. A. Scott, assaying the insulin content of an islet cell tumor provided by W. R. Campbell and R. R. Graham, found a value of 214 units per gram of islet tumor tissue (total weight of tumor 3.3 grams). This concentration is not greatly different from the calculated value for the *islet* tissue of normal human and rat pancreas. The concentration is approximately that which could be expected if the tumor were pure islet tissue.

Zinc and insulin content. Because of the association of zinc with insulin crystals and with commercial preparations of insulin, and because of fibrotic changes in the pancreas resulting from zinc feeding (72), Scott and Fisher (16) undertook to determine if there were any significant relation between the insulin and zinc content of the pancreases of rats maintained on basal diets and diets containing added zinc. They found that the ingestion of large amounts of zinc caused an increase in the total zinc content of the pancreas but no significant change in the total insulin content of the pancreas (basal 11.52 units, zinc diet 10.40 units). It is interesting to note, however, that the average *concentration* of insulin in the pancreas was considerably higher in the group given zinc (basal 1.68 units per gram, zinc diet 2.56 units per gram), but the weight of the pancreas was only about half that of the control group. This illustrates the importance of the way in which results are expressed. If these workers had presented their results only in terms of concentration of insulin per gram of pancreas, an erroneous conclusion concerning changes in insulin content might have been reached because of the great reduction in the acinar tissue.

DIET AND THE INSULIN CONTENT OF THE PANCREAS. Some of the most interesting changes in the insulin content of the pancreas are those related to alterations in the diet. Most of these concern the effect of diminishing the caloric intake or reducing the amount of carbohydrate ingested. Certain other dietary factors, however, have been investigated.

Fasting and undernutrition. One very definite change in the insulin content of the pancreas is that produced by fasting. Best, Haist and Ridout observed that in rats starved for seven days the insulin content of the pancreas was much

lower than in control animals with the same initial weight (23) (73). The weight loss in the fasted rats was 23 per cent of the initial body weight; the insulin content for these animals was 14.1 units per group of 10 rats and that for the controls, 26.5 units per group. Reducing the intake of a balanced diet in male rats was found by Haist and Best (63) to bring about a reduction in the insulin content of the pancreas. With a daily intake of 15 calories per 225 gram rat the results were practically the same as for complete starvation. With an intake of 45 calories the value was nearer the control level though, on the average, less than for the group receiving 65 calories per 225 gram rat. Undernutrition itself thus may influence the insulin content of the pancreas, though these data do not rule out the possibility that even when using different amounts of a balanced diet the available carbohydrate may be the limiting variable.

In dogs the effect of fasting is more difficult to demonstrate than in rats. The animals do not form as homogenous a group as the rats, and the equivalent fasting period is much longer. Haist, Campbell and Best (74) report that in dogs fasted for nine days the insulin values were in the lower part of the normal range. Aubertin, Lacoste, Saric and Castagnou (75) claim that fasting increased the islet volume in dogs and that in two dogs fasted for 15 and 43 days the insulin concentration in the pancreas was elevated. Since the normal values they give, however, (0.15 to 0.23 unit per gram of pancreas) are about one-tenth of those commonly reported and since the normal variation in dogs is great, their results may be questioned. According to Foglia (76) the pancreases from dogs fasted 2 to 3 weeks when grafted into the necks of depancreatized dogs showed no impairment of the ability to reduce blood sugar in four cases, a slightly diminished ability in one and a very reduced ability in two others. These findings in general correspond with the results of insulin assay in dogs and might be a reflection of the insulin concentration in beta cells. (See the discussion in the section on the pituitary gland.)

Fat, sugar and protein. Feeding a diet very rich in fat (90 per cent fat + agar, salt mixture and vitamins) gives a reduction in the insulin content of the pancreas comparable to that obtained with fasting (23) (73). Some of our unpublished data indicates that this effect of fat-feeding on insulin content is still obtained if fat in the form of olive oil is given by stomach tube in amounts equivalent to the normal total caloric intake. The effect is obtained also even though the accumulation of liver fat is prevented by the daily administration of 100 mgm. of choline chloride per rat. Best, Haist and Ridout (23) previously reported that in rats poisoned with carbon tetrachloride the insulin content of pancreas was not related to the level of liver fat. The effect of fat-feeding on insulin content therefore cannot be ascribed merely to a poor caloric intake or to an altered liver function associated with the accumulation of fat.

A diet moderately rich in fat (fat 46 per cent, protein 15 per cent, carbohydrate 28 per cent by weight) appears to give a slight decrease in the insulin content of the rat pancreas, but the findings do not suggest that an extensive depletion of insulin would be produced readily by a diet of this type (23). The demonstration of the effect of fat-feeding in dogs is accompanied by the same difficulties

as the demonstration of the effect of fasting. Haist, Campbell and Best (74) report values in the lower part of the normal range for 3 dogs fed fat for eight days.

Since fasting and fat-feeding give similar reductions in insulin content the possibility exists that the lowering of content in both instances results from a decreased supply of carbohydrate. Best, Haist and Ridout (23) showed that when equicaloric rations of sugar and fat were given, the insulin content of the pancreas remained higher in the sugar-fed animals than in those fed fat. The difference amounted to 42 per cent of the higher value despite the fact that the weight loss was comparable in the two groups. There was a noticeable reduction in insulin content in the sugar-fed group, due probably to a reduced caloric intake. Feeding a diet moderately high in carbohydrate for a period of 3 to 7 months gave no appreciable change in insulin content (23). Other observers confirm this finding that high carbohydrate diets do not greatly affect insulin content (77). A diet composed almost entirely of protein appears to have an effect on insulin content intermediate between that of carbohydrate and that of fat (23). Some of our unpublished data indicate that rats receiving a diet in which gelatine is the protein have a lower content of insulin in the pancreas than those receiving equicaloric rations of the same diet with casein replacing the gelatine. The diet was otherwise balanced. Four "casein" groups gave an average content of 21.6 units per group of 10 rats, whereas four "gelatine" groups receiving the same caloric intake had an average content of 15.2 units per group of 10 rats. In all instances the animals receiving the gelatine diet lost weight while those on the casein diet gained weight. It is possible that any diet not adequate for growth or maintenance would give similar results and that it is not a specific effect for a particular amino acid deficiency.

Carbohydrate tends not only to maintain the level of insulin content but also to restore the level following a period of starvation. When rats are starved for 7 days and then given a balanced diet, the insulin content of the pancreas returns to normal within 7 days. When they are given a diet of sugar the content is brought back within the normal range but the level is not maintained. Fat leads to no elevation in content above the level in the fasting animal but, if anything, causes a further reduction (23) (73).

Casein has an effect similar to sucrose when fed to rats previously starved for 7 days. At the end of 7 days of re-feeding the value for the sugar-fed rats averaged 19.8 units per group of 10 rats, and for the casein-fed 18.3 units per group. In two experiments in which dextrose was given in amounts equivalent to 50 per cent of the casein intake, the casein groups averaged 20.3 units per group of 10 rats, and those receiving dextrose 17.7 units per group (unpublished data).

The data already presented would justify the conclusion that the effect of diet on the insulin content of the pancreas is due in large part to the amount of available carbohydrate in the diet, although certain other factors are not excluded.

Insulin, fasting and fat-feeding. In an attempt to throw some light on the

way in which the effect of fasting or fat-feeding was brought about, insulin was injected into fasting and fat-fed rats (13) (78). The insulin administration enhanced the effect of fasting and fat-feeding, causing a reduction in the insulin content of pancreas to still lower levels. Best and Haist conclude "it is improbable that the insulin liberation by islet cells would be increased when insulin is given. The insulin production by the islet cells is presumably reduced. Since insulin administration enhances the effect of fasting or fat-feeding, the effects of insulin administration, fasting and fat-feeding are similar. These procedures, i.e., insulin administration, fasting and fat-feeding, apparently rest the pancreatic islet cells by reducing the need for endogenous insulin." The insulin-treated, fasted and fat-fed rats which have a low insulin content of the pancreas, show a *reduction* in the specific granules of the beta cells of the islets of Langerhans (11) but no evidence of hydropic degeneration. This further emphasizes the close relation between the specific granulation of beta cells and insulin content. The influence of fasting and fat-feeding in preventing the effects of diabetogenic anterior pituitary extracts on pancreatic insulin content (74) and in alleviating the effects of extensive partial pancreatectomy (6) provides further evidence that these procedures "rest" the islet cells. Whether or not in normal animals fasting, fat-feeding and insulin administration leave the islet cells with a greater functional reserve, a greater potentiality for increased function without damage, cannot be decided at present.

Endocrines, fat-feeding and fasting. Himsworth and Scott (83) contend that fat-feeding and fasting influence sugar tolerance and insulin sensitivity by increasing the activity of the pituitary gland. Chambers, Sweet and Chandler (84) found, however, that starved hypophysectomized dogs showed a definite hyperglycemia and an absence of rise in respiratory quotient following glucose administration. That is, they responded in a manner similar to normal animals and hence the pituitary probably is not involved in the phenomenon. The pituitary also does not seem to be concerned with the effect of dietary changes on the insulin content of pancreas. The insulin-lowering effect of fat-feeding can still be obtained after the removal of the pituitary gland (13) (85) and, in the hypophysectomized animal, after the insulin content of pancreas has been reduced by the feeding of fat it can be restored by giving a balanced diet (13) (85). The reduction in insulin content by fat-feeding can be obtained also in adrenalectomized and gonadectomized rats (25) (86). These findings indicate that the pituitary, adrenal glands and gonads are not fundamentally involved in the influence of fat-feeding on the insulin content of the pancreas. The pancreas appears to be able to regulate production and liberation according to the need for insulin in the absence of these glands.

Relation to other observations on diet and carbohydrate metabolism. When an animal is starved or when certain diets are given, definite changes in the response of the body to administered carbohydrate become evident. There is a progressive decrease in sugar tolerance, especially marked in the intermediate period and improving again in the final stage of fasting. This decreased tolerance is accompanied by excretion of sugar in the urine and an absence of the normal rise

in respiratory quotient after glucose administration. For the relevant literature concerning these changes the reader is referred to a comprehensive review by W. H. Chambers on Undernutrition and Carbohydrate Metabolism (79). Haist, Campbell and Best (74) discuss the relation of the changes in insulin content to these observations. They suggest two possibilities: *1*, that when the islet activity is reduced, the immediate response of islet cells to any sudden increase in the need for insulin is less than normal, or *2*, when the level of insulin liberation is low, that is, when the continuous outpouring of the hormone is reduced, the tissues and especially the liver are altered so far as their response to sugar is concerned. A third possibility is that even though the low insulin content is associated with conditions causing the above phenomena, islet function is not involved in the phenomena. Soskin (12) cites evidence to indicate that "in the presence of a sufficiency of insulin, but not necessarily an extra secretion from the pancreas, the normal liver, as one of its responses to administered dextrose, decreases the output of blood sugar which it has been previously supplying from its own resources." Soskin, Essex, Herrick and Mann (80) were able to show by direct measurements that the liver played an important rôle in the regulation of blood sugar level by taking up sugar from the blood or liberating sugar into the blood as needed. Hence the response of the liver to the blood sugar level would seem to be a very important factor in sugar tolerance. For a discussion of this subject see the review by Soskin (12). Soskin considers that insulin helps to determine the threshold of the homeostatic mechanism of the liver, i.e., the level at which the hepatic inhibitory response is obtained. He does not show, however, that with a lower than normal continuous insulin supply no change in sugar tolerance results. In human diabetics and depancreatized dogs, Ricketts (81) obtained diabetic tolerance curves in response to breakfast despite the fact that a dose of protamine insulin given on the previous evening was sufficient to keep the blood sugar normal through the following morning if no food were taken. Others report results at variance with these (12) (82). Soskin suggests that an extra secretion of insulin ordinarily resulting from hyperglycemia, while not essential, probably acts as a factor of safety in increasing the efficiency of blood sugar regulation (12). While the rôle of the pancreas in the control of the blood sugar level is controversial, it seems fair to conclude at least that physiological changes in islet function may lead to quantitative changes in the blood sugar regulation.

The effect of vitamin deficiency. Very little work concerning the effect of vitamin deficiency or excess on the insulin content of pancreas has been reported. Best, Haist and Ridout showed that rats receiving a diet complete except for certain vitamins, and containing vitamins A and D in the form of cod-liver oil concentrate had a reduced insulin content of pancreas, probably due to the diminished caloric intake. A second group fed the same caloric intake but having vitamin B_1 added to the diet had an insulin content of pancreas not significantly different from the first. The addition of B_1 thus seemed to be without effect on insulin content (23). Further systematic studies in this field should be undertaken.

ENDOCRINES AND INSULIN CONTENT. While there is at present no evidence that the endocrine glands exercise any *fundamental* normal control over the insulin content of the pancreas, yet in certain instances preparations obtained from them do greatly alter the insulin content. For that reason it is important to know which glands affect the insulin content of pancreas and under what circumstances the influence is noted.

Adrenals. The adrenal glands can be removed without greatly altering the insulin content of pancreas. Fraenkel-Conrat, Herring, Simpson and Evans (77) in one experiment reported that the pancreases of adrenalectomized rats contained more insulin than unoperated rats, but Haist and Bell (25) (86) found that adrenalectomized rats maintained on sodium chloride showed no significant change in the insulin content of pancreas as compared to control animals receiving the same caloric intake (paired-fed controls, 12.4 units per group of 10 rats; adrenalectomized, 12.5 units per group of 10 rats). It has been reported also that the administration of adrenal cortical extract gives little change in the insulin content of pancreas (86) (77). The finding that the diabetic state of partially depancreatized rats can be increased by certain adrenal steroids (87) (88) and the report that glycosuria, though not true diabetes, can be produced in normal rats by the administration of 17-hydroxy-11-dehydrocorticosterone (89) make it seem possible that if certain specific adrenal steroids were used in sufficient quantities, some measurable effect on insulin content might be evident. Fraenkel-Conrat et al. (77) observed that adrenocorticotrophic pituitary preparations increased the insulin content of rat pancreas. If this were due to the adrenocorticotrophic effect then an adrenal influence on insulin content would be indicated. They believe, however, that the effect was the result of contamination with lactogenic hormone. The fact that the insulin-reducing effect of fat-feeding and the restoration of insulin content by a balanced diet can be obtained after adrenalectomy is good argument for a normal regulation of pancreatic insulin that is independent of the adrenal glands (25) (86).

Gonads. Sex differences in the insulin content of pancreas are not striking. Fraenkel-Conrat et al. (77) reported a higher average insulin content in normal female than in male rats, though there was great overlapping in the range of values. This difference was not observed after hypophysectomy. The data of Haist and Bell (25) indicate that, considered over a wide range of body weight, males and females have roughly the same insulin content of pancreas. There are differences, however, in the effect of certain procedures on the insulin content in the two sexes. For example, horse pituitary extract is pancreatrophic in the female but not in the male rat unless the gonads are removed (90). The insulin content of the pancreas is not significantly altered by removal of the gonads and the insulin-reducing effect of fat-feeding can still be obtained in their absence (25). However, certain materials obtained from the gonads or simulating their effects can cause a great change in the insulin content of pancreas.

Griffiths and Young (91) report that implantation of tablets of oestrogen in rats interferes with normal pituitary function and depresses growth. The pancreas is not reduced in proportion to the body as a whole and the pancreatic

insulin is significantly increased in relation to the body weight. An elevation in the insulin content of the pancreas in rats is reported to result from the administration of the oestrogens, oestrone (92), oestriol (93), oestradiol (93) (94), oestradiol dipropionate (95) and stilboestrol (93) (94). The insulin content of the rabbit pancreas is increased by hexoestrol (34) (96). Progesterone has no insulin-increasing effect (94) and the androgen testosterone does not induce an elevation in the insulin content of pancreas in rats (93). In fact, some claim that the content is decreased by testosterone (94). Griffiths reported that alpha methyl stilbene, closely related to hexoestrol and stilboestrol, greatly increased the pancreatic insulin in the rabbit but was non-oestrogenic (34) (96). The increase with this material was tremendous (control, 5.2 units per gram; injected, 24.5 units per gram). Since the rabbit pancreas is diffusely distributed and since only portions of the pancreas and not the whole gland were used for the determinations, serious error might arise from this source. Because the alpha methyl stilbene was not oestrogenic Griffiths concluded that the insulin-increasing properties of hexoestrol and stilboestrol "have little to do with their oestrogenic properties." Fraenkel-Conrat and associates (95) showed that after hypophysectomy the insulin-increasing effect of oestradiol dipropionate was not obtained. The insulin content of the pancreas was elevated by the implantation of pituitary glands obtained from rats receiving oestrogens, while pituitaries from control rats did not have this effect. These experiments suggest that the pituitary mediates the insulin-increasing activity of oestrogens.

Vazquez-Lopez (97) gives as histological evidence of excessive secretory activity of the islet cells after injections of oestrogens the demonstration of marked enlargement of the Golgi apparatus, and harmonizing with the results on insulin content are the reports that injections of oestrin cause an increase in the islet tissue in the pancreas of certain species (98) (99). Castration has been reported to lead to hyperplasia of islet tissue in guinea pigs, though the administration of testosterone apparently had no effect on pancreatic morphology (100). Others contend that, in the dog, injections of testosterone propionate lead to a reduction in islet tissue (101). The histological effects of testosterone seem to be no more definite than the effects on pancreatic insulin. Owing to the fact that the islets vary greatly in size and number among members of the same species, erroneous conclusions may be drawn from estimates of the effect of injected materials on the islets unless some good quantitative measure of islet volume is used. Even when this is done the volume of the beta cells, which is the important value, still remains undetermined.

Thyroid. Various claims have been made concerning an interrelationship of the thyroid gland and the pancreas. Fraenkel-Conrat and associates (102) emphasize the influence of dosage on the effect of thyroxin; for example, with low doses thyroxin synergizes with growth hormone while with high doses weight loss occurs. These workers tried the effect of thyroxin in hypophysectomized rats, giving doses sufficient to restore the oxygen consumption to normal. No weight loss occurred, but the insulin content of the pancreas was reduced to about half that of the controls. In one experiment performed on normal rats,

the administration of thyroxin led to an increase in pancreatic insulin. Some observers have reported that the injection of thyroxin and thyroid extract into normal animals gives hyperplasia of the islets (103) but this was not confirmed by others (104).

Pituitary. A relationship between the anterior pituitary gland and the *pancreatic islets* had been suspected because of the high incidence of diabetes in certain conditions associated with pituitary dysfunction (105). Johns, O'Mulvenny, Potts and Laughton in 1927 (106) reported that hyperglycemia, glycosuria and polyuria had been produced in dogs by injecting extracts of the anterior pituitary gland. Injections of anterior pituitary extracts were shown to produce diabetes in intact experimental animals by Evans, Meyer, Simpson and Reichert (107), Baumann and Marine (108) and Houssay, Biasotti and Rietti (109). Evans and associates noted a persistence of the diabetic state after the administration of the extract was discontinued, but the significance of these findings was not generally appreciated until F. G. Young showed conclusively that if the course of injections of anterior pituitary extracts was sufficiently severe and prolonged, the diabetes persisted indefinitely after the injections were discontinued (110). The pancreases in these "permanently" diabetic animals contained fewer than normal islets (111) (112) (113) (114) (115) (116). Granule staining showed that the lack of granular beta cells was almost complete (8) (9) (111) (115). Campbell and Best (112) reported that the insulin content of the pancreas of the permanently diabetic dog was extremely low (less than 2 units per total pancreas), a finding that was later confirmed (7) (117).

Best, Campbell and Haist (7) observed that during the course of the injections of the anterior pituitary extract, before the diabetic state had become "permanent," there was a progressive reduction in the insulin content of the pancreas. When, after 7 daily injections, the administration of the extract was discontinued, the insulin content of the pancreas returned to normal. If the course of injections of the extract was sufficiently severe, then cessation of the injections was not followed by restoration of the insulin level in the pancreas, the content remaining low for as long as 198 days. Ham and Haist (8) (9), reporting on the histological changes in the pituitary-injected animals, showed that there was a progressive degranulation of the beta cells of the islets of Langerhans that paralleled the fall in insulin content. The degranulation was followed by hydropic degeneration of the beta cells. With the cessation of the injections of anterior pituitary extract the beta cells regained their normal granulation, the return of granulation corresponding to the restoration in insulin level (9). Richardson and Young (111) had previously reported cellular proliferation and hydropic degeneration of the beta cells of the islets in 2 dogs during the course of anterior pituitary injections.

The islet changes in the pituitary-injected dogs closely resemble those reported by Homans (5) and Allen (6) for partially depancreatized animals. Also, the fall in insulin concentration in the pancreatic remnant in partially depancreatized animals is similar to the change in insulin level found in the animals receiving injections of anterior pituitary extracts. Allen (6) concluded, and Bell, Best

and Haist (14) support the view, that the islet changes in the partially depancreatized dog are due to overwork of the beta cells of the islets with consequent exhaustion. Haist, Campbell and Best (74) discuss the cause of the alterations in the animals with pituitary diabetes and conclude that here too the islet changes result from an exhaustion through overwork. In these animals the overwork probably results from several different influences. The diabetogenic pituitary extracts have profound extra-pancreatic effects. They can exhibit their hyperglycemic and glycosuric action in the absence of the pancreas (118) (119) (120) and the action of insulin is antagonized even before the blood sugar is elevated (121) (122) (123). Many different trophic principles may be involved since organs and tissues other than the pancreas show cellular proliferation and evidence of increased activity while the extracts are being given (8) (9). Best, Campbell and Haist (7) report an interesting inverse relationship between the fasting blood sugar level and the concentration of insulin in the pancreas of pituitary-injected dogs. When the injections of the anterior pituitary extract were discontinued, the blood sugar fell rather quickly to normal values but the insulin content of the pancreas remained low until after the normal blood sugar level was restored. These findings seem to suggest that the blood sugar level, or some extra-pancreatic factor causing the elevation in blood sugar, is the cause of the islet changes rather than the reverse. The experiments are not conclusive in this regard and these authors *do not assume* that the elevated blood sugar level is the main factor stimulating insulin secretion. It seems reasonable to conclude, however, that as a result of the extra-pancreatic effects of the extract the need for insulin is increased. The fact that the effects of anterior pituitary extract on insulin content and pancreatic islets can be prevented to some extent by procedures such as the administration of insulin (10) (124) (125), fasting and fat-feeding (74), which presumably reduce the need for endogenous insulin, argues in favor of the view that the effects of pituitary injections on islets are due to overwork. Ham and Haist (9) suggest, however, that in addition to increasing the need for insulin, the anterior pituitary extracts may remove restraints which ordinarily hold secretory and growth activities within certain limits. Hence a direct effect on the islets may operate along with extra-pancreatic influences by permitting or encouraging the excessive overwork which leads to exhaustion. Marks and Young (17) also speculate on the manner in which the pituitary effect is brought about.

Houssay and his associates (126) (127) grafted the pancreases of dogs exhibiting pituitary diabetes into the necks of depancreatized animals. They concluded on the basis of the effect on blood sugar that there was a diminished liberation of insulin from these pancreases. The conclusion that there is a diminished capacity for secretion in these pancreases seems reasonable. However, it is difficult to evaluate the results. Since the pancreatic transplants have a limited life one might wonder whether or not some deterioration of the tissue may begin at the time the transplant is made. Since insulin can be extracted from the pancreas by perfusion (128) it is possible that insulin is being washed out of the pancreatic transplant by the blood of the recipient animal.

The amount washed out would depend upon the insulin concentration in the islets and the relation of this concentration to the blood insulin level. Such a test, then, might merely reflect the insulin content of the transplanted pancreas. However, it does seem likely that once hydropic degeneration occurs or permanent diabetes with islet atrophy has been established, the liberation of insulin by the pancreas is decreased, and it probably is reduced before this time when the content of insulin is low and marked degranulation of the islet cells is evident.

It is not within the scope of this paper to discuss fully the nature of the pituitary diabetogenic factor or factors. A marked reduction in the insulin content of the pancreas is associated with the diabetic effects in the dog (7) (112) (129) (17), and a loss of diabetogenic activity is accompanied by an inability of the extracts to reduce the insulin content (17). Crude saline, alkaline and acid extracts of fresh anterior pituitary glands (130), picric acid preparations (131), pseudoglobulin (17) (132) and globulin fractions (7) (133), growth (107) (108) (134) (135) and ketogenic (133) (134) preparations have been shown to be diabetogenic in dogs.

The diabetogenic factor closely accompanies the growth factor in its preparation, and Shipley and Long (134) suggest that the ketogenic, growth and diabetogenic factors may be identical. Young (136) observed also that pituitary diabetogenic preparations usually have growth-promoting activity. However, Marks and Young (17) found that "stale" crude extracts of fresh pituitary glands retain growth-promoting activity but are not diabetogenic and they conclude that the diabetogenic and growth-promoting substances are not identical. Fraenkel-Conrat and associates (77) found that the administration of growth hormone brought about a decrease in the insulin content of the pancreas in the rat, but they were unable to produce diabetes by its administration. This effect of growth hormone was still obtained after adrenalectomy but not after removal of the pituitary. The importance of the growth factor in the diabetogenic action is again emphasized in the recent work of Marx, Anderson, Fong and Evans (136a), who report that purified growth hormone preparations practically free from lactogenic, adrenocorticotrophic, thyrotrophic and gonadotrophic factors greatly increase the urinary excretion of glucose in partially depancreatized, sugar-fed rats.

Albumin and euglobulin fractions of anterior pituitary are not diabetogenic (132) and the diabetogenic effect apparently is not produced by relatively purified preparations of prolactin (133) (134) (137) (138), thyrotrophic (138), gonadotrophic (136) (139) or glycotropic (136) factors. Since Houssay and associates showed that the diabetogenic action can be obtained in some species in the absence of thyroid, gonads and adrenals (140) (141), it is difficult to see how a pituitary principle acting on these glands separately could be responsible for the total diabetogenic activity. The adrenal gland, however, does seem to be involved in some way in the diabetogenic effect. Long and Lukens (142) (143) found that after removal of the adrenals the diabetogenic effect of pituitary extracts was not obtained in cats. The diabetogenic effect has been observed by Houssay and Biasotti in partially depancreatized, adrenalectomized dogs

maintained in good condition with cortical extract (144), desoxycorticosterone or sodium chloride (141). It would seem then that the adrenals are important in the production of the diabetogenic effect, though some effect not mediated by the adrenals must also be granted (141). Of all the organs in the body, the liver is most necessary for the diabetogenic effects of pituitary extracts (107) (141) (145). The occurrence of diabetes as a result of pituitary injections probably depends not on a single pituitary factor but on a variety of effects, many of them "trophic" in nature (9), exerted on a number of different organs and tissues of the body. Long (146) considers the possibility that there may be two constituents of the diabetogenic factor, one, heat-stable, able to cause glycosuria in a hypophysectomized-depancreatized animal, and one, heat-labile, required to give ketonuria and glycosuria in the intact animal. Young (136) concurs in this view and suggests that the heat-stable factor may be responsible for the extra-pancreatic effects.

Certain pituitary preparations causing a reduction in the insulin content of pancreas in the dog occasion an increase in the insulin content of pancreas in the rat (17) (33) (77) (92) (94) (129). Anselmino, Herold and Hoffmann (147) had reported that following the injection of an anterior pituitary extract there was an increase in the size of the islets of Langerhans. Some confirmed, but others were unable to repeat their results. Richardson and Young (30) showed, however, that injections of crude extracts of the anterior pituitary gland caused an increase in the volume of the islets in Wistar rats. The increase in the insulin content of pancreas that results from the injection of anterior pituitary extracts in rats might be expected then, in view of the increase in islet volume. Marks and Young (17) state that evidence is lacking to show that the insulin-increasing and islet-increasing factors are the same, but intimate that until evidence for a plurality of the factors is forthcoming, the provisional assumption may be made that one substance is responsible for both effects.

Signs of proliferation are evident in the islets of dogs as well as of rats injected with anterior pituitary extracts. Richardson and Young (111) were the first to note evidences of cell division in the islets of Langerhans in pituitary-injected dogs. Ham and Haist (8) (9) found proliferation not only of the islet cells but also of acinar cells and the "mother cells" of the small ducts of the pancreas in dogs receiving pituitary injections. Even in the dog, then, there are some proliferative effects of the extracts, though here the degranulation and degeneration seem to predominate and the insulin content of the pancreas falls.

The mouse resembles the rat, showing an elevation in the insulin content of pancreas when crude extracts of pituitary are used (26). The rabbit exhibits a fall like the dog, though usually not so great (26) (131).

Considerable work has been done in an effort to separate the diabetogenic (dog) and insulin-increasing or pancreatrophic factors (rat). Marks and Young (17) showed that a relatively purified diabetogenic, psueudoglobulin, fraction of anterior pituitary extract resembled a crude extract in having diabetogenic, insulin-increasing and growth-promoting effects. "Stale" crude extracts of fresh anterior lobe tissue (extracts which had been standing 24 hrs. at room tem-

perature or which were incubated for 5 hrs. at 37°C.) were found to have little or no diabetogenic effect, though they did have growth-promoting and insulin-increasing activity. Extracts of commercial acetone-desiccated anterior pituitary glands with no detectable diabetogenic or growth-promoting activity were shown to increase the insulin content of the rat pancreas. While Marks and Young felt that the insulin-increasing (in rats), diabetogenic (in dogs) and growth-promoting substances were not identical, they were unable to obtain diabetogenic fractions free from growth-promoting or insulin-increasing activity, or to prepare growth-promoting extracts free from insulin-increasing activity. They state that their observations "do not preclude the possibility that the anterior lobe tissue contained a substance (pseuglobulin?) having all three activities (diabetogenic, growth-promoting and insulin-increasing), and that differential inactivation of prosthetic groups concerned in the different activities may take place." Later in the paper, however, they conclude that "there are certainly two principles."

Funk and associates (94) found that a protein fraction, prepared by alkaline extraction of anterior pituitary glands previously extracted with 10 per cent salt solution, increased the insulin content of pancreas. Fraenkel-Conrat and associates (77) report that unfractionated alkaline anterior pituitary extracts only increased the insulin content of pancreas in excessive doses. Globulin fractions of these extracts had no effect. Their report concerning a decrease in the insulin content of pancreas in the rat when growth hormone was administered has been mentioned previously. This observation supports the contention of Marks and Young (17) that the growth-promoting and insulin-increasing factors are not identical.

Marks and Young (92) conclude that the insulin-increasing factor is not identical with the gonadotrophic principle. First, ox prolactin preparations which contained only a trace of gonadotrophic principle had insulin-increasing effects. They found, moreover, (90) that horse pituitary extracts which were actively gonadotrophic did not elevate the insulin content of pancreas in male rats, though they were active in females. Ox pituitary extracts, which were only moderately gonadotrophic, gave an elevation in the insulin content in both male and female rats. In this connection they showed that treatment with testosterone inhibited the insulin-increasing action of ox pituitary extract in male rats. Since they found that extracts of horse pituitary actively increased the insulin content of the pancreas in the female but not in the male animal they considered that the effect of the endogenous testosterone might explain the failure of horse pituitary (actively gonadotrophic) to influence the insulin content of pancreas in male rats. Moreover, both the ox and the horse anterior pituitary extracts had an insulin-increasing effect in gonadectomized male and female rats. This, plus the fact that testosterone itself did not induce a rise in insulin content, led them to conclude that the gonads do not mediate the pituitary effect (92). The work of Fraenkel-Conrat et al. (95) would indicate that, on the contrary, the pituitary probably mediates the insulin-increasing activity of certain gonadal substances (oestrogens).

Marks and Young (92) found that prolactin was very effective in increasing the insulin content of the pancreas in the rat but considered that it was improbable that prolactin and the pancreatrophic principle were identical. They thought this for several reasons. First, pseudoglobulin fractions of ox pituitary had insulin-increasing effects and yet they were free from detectable prolactin. Secondly, the daily dose of pituitary extract required to give an insulin-increasing effect contained only $\frac{1}{5}$ of the effective daily dose of prolactin. Finally, sheep pituitary extracts were only half as potent as ox pituitary extracts in giving the increase in insulin content, and yet the sheep pituitaries were richer in prolactin. Fraenkel-Conrat and associates (77) found that lactogenic preparations which were chemically pure, as judged by electrophoretic, ultracentrifuge and solubility studies, increased the insulin content of pancreas. The lactogenic preparations were effective in 3 out of 5 tests on normal animals when 1 mgm. or more daily was given for at least 17 days, and were similarly active in hypophysectomized rats. Adrenocorticotrophic preparations which were also effective in increasing insulin content were contaminated with lactogenic hormone up to 25 per cent and these workers felt that the effect was probably due to the contaminating lactogenic hormone. Funk and associates (94) report that highly purified prolactin reduces the insulin content of rat pancreas, but Fraenkel-Conrat et al. (102) were unable to confirm this effect, finding only an increase when pure lactogenic hormone was used under the conditions reported by these workers.

Marks and Young (92) conclude that the thyrotrophic factor is not responsible for the increase in insulin content, since only traces of it are present in the prolactin fraction which is effective in that regard. Fraenkel-Conrat and associates (77) showed that a purified thyrotrophic preparation was not active in elevating pancreatic insulin. The fact that thyroxin itself lowers the insulin content of pancreas in hypophysectomized rats (102) may also be taken as evidence against the view that the thyrotrophic principle is the responsible factor. The material causing the increase in insulin content is relatively unstable to heat and, according to Marks and Young (92), loses most of its activity when heated to 100°C. for a short time. This serves to differentiate it from the glycotropic factor which is relatively heat-stable. They found also that alcohol-dried pituitary and an alcohol-soluble fraction of ox pituitary were not significantly pancreatrophic when injected or given by mouth.

The results of experiments on hypophysectomized animals assist in the evaluation of the rôle of the pituitary in the normal control of islet activity. Chambers, Sweet and Chandler (84) reported that the insulin content of the pancreas in hypophysectomized dogs did not differ from that of normal animals. Haist and Best (13) (85) showed that while the insulin content of the pancreas in hypophysectomized rats was slightly less than that of control animals fed ad libitum, it did not differ significantly from that of control animals receiving the same caloric intake. Fraenkel-Conrat and associates (77) also observed that there was no significant difference between the insulin content of normal and hypophysectomized female rats. In 80 gram hypophysectomized rats, Griffiths

and Young (24) (91) report an increase in the insulin content of pancreas, but in hypophysectomized rats of 100 grams or more the amount of insulin per 100 grams of body weight remains virtually the same as for normal controls. According to Krischesky (148) the ratio of islet tissue to body weight in the *male* rat is increased by hypophysectomy. Haist and Best (13) (85) found that in some respects at least hypophysectomized rats respond like control animals, since they exhibit a similar reduction in the insulin content of pancreas when fat is fed. In these animals the low values following fat-feeding can be brought back to normal by feeding a balanced diet. It appears that the pancreas can regulate the production and liberation of insulin according to the need for it, in the absence of the pituitary gland (85). This suggests that the pituitary exercises no *fundamental* normal control over pancreatic islets, despite the effects of injected extracts of that gland.

Removal of the hypophysis alters the effect of injections of pituitary materials. Crude saline extracts of the anterior pituitary gland have a less striking insulin-increasing effect in hypophysectomized than in normal rats (33). Griffiths and Young (24) (91) indicate that injection of fresh and "stale" anterior pituitary extracts in hypophysectomized rats will induce growth and lead to an increase in the insulin content of pancreas which is approximately proportional to the increase in body weight. Griffiths (24) concludes that "although the pituitary gland controls the increase in pancreatic insulin during growth, it may not do so by means of a specific insulin-increasing hormone but rather the increase is associated with the general increase in constituents involved in somatic growth." Fraenkel-Conrat and associates (77) suggest, however, that the existence of opposing balanced influences (insulin-increasing and insulin-decreasing) of the pituitary may afford an explanation of why neither removal nor implantations of the gland produce effects on the pancreatic insulin.

From the reports already referred to it will be evident that many anterior pituitary preparations increase the insulin content of pancreas in the rat. Crude saline extracts, crude "fresh" alkaline extracts, "stale" alkaline extracts, alkaline extracts of commercial acetone-desiccated glands, pseudoglobulin fractions, alkaline extracts of glands previously extracted with sodium chloride, adrenocorticotrophic and lactogenic preparations are all reported to have this effect. In addition to this, the insulin content of pancreas can be elevated in intact rats by the injection of thyroxin or by the administration of the oestrogens, oestrone, oestriol, oestradiol, stilboestrol and oestradiol dipropionate. Some of these preparations have been shown to have their significant effect only in the presence of an intact pituitary gland. Injections of crude saline and alkaline extracts of anterior pituitary and of the oestrogen, oestradiol dipropionate, do not lead to the usual well-marked elevation in insulin content of pancreas in the absence of the pituitary. The lactogenic and adrenocorticotrophic pituitary preparations, however, apparently are effective in hypophysectomized rats. The fact that, in the absence of the pituitary, certain preparations are not insulin-increasing, whereas in the presence of the pituitary they do have this effect, would indicate that the insulin-increasing action may be mediated or potentiated by the anterior pituitary gland.

If there is a factor having a direct insulin-increasing action, it must be demonstrated that the effect of this preparation on the islets is not mediated by the pituitary, thyroid, adrenals or gonads. It must also be shown that the pancreatic changes are not the indirect result of extra-pancreatic effects of the preparation. The purified lactogenic and adrenocorticotrophic materials which effectively increase insulin content in the absence of the pituitary come closest to meeting these requirements, but as yet no preparations satisfy all of them.

As far as the insulin content is concerned the effects of the different pituitary preparations already reported are *1*, a great reduction in the insulin content of pancreas in the dog and rabbit; *2*, an increase in the insulin content of pancreas in the rat and mouse; *3*, a decrease in the insulin content of pancreas in the rat. The reduction in the insulin content of pancreas in the dog is associated with diabetes and the extracts producing these effects are said to be diabetogenic. A reduction in the insulin content of pancreas without accompanying diabetes does not constitute a diabetogenic effect. The influence on growth and secretion in the pancreas is called the pancreatrophic effect. The same extract causing diabetes and a reduction of pancreatic insulin content in the dog may cause islet cell proliferation and an increase in the insulin content of pancreas in the rat.

Marks and Young (17) discuss the possible reasons for the difference in the response of the rat and the dog. They state that in the extracts "there are certainly two principles (diabetogenic and insulin-increasing), and the observed effects can be provisionally explained on the assumption that the dog is more sensitive than the rat to one, and the rat more sensitive than to the dog to the other." The extra-pancreatic mechanism may be more sensitive in the dog and the influence of the insulin-increasing factor may be exerted more readily in the rat. Ham and Haist (9) point out that secretion as well as growth is stimulated by many if not all of the so-called pituitary "trophic" principles. They argue that a pancreatrophic factor, if it acted like other "trophic" materials, would (directly or indirectly) stimulate secretion as well as growth. The same principle then may be involved in the effects on the islets in dogs and in rats. However, if the finding is confirmed that globulin fractions and growth preparations (77), which are diabetogenic in the dog, do not elevate the insulin content of pancreas in the rat, then this would consititute evidence in favor of separate diabetogenic and insulin-increasing factors.

It seems reasonable to conclude that while many extracts or fractions which are diabetogenic in the dog are insulin-increasing in the rat, the reverse is not true. Many of the insulin-increasing preparations are not diabetogenic. This may be due to the necessity of a much more potent solution for the diabetogenic effect than for the insulin-increasing action. It is possible that the diabetogenic action results from a combination of many extra-pancreatic influences, which increase the need for insulin and indirectly stimulate the islets, along with a direct effect (pancreatrophic ?) which permits or encourages excessive secretion to occur (9). If the pancreatrophic factor is involved one might postulate that new islet cells would be formed. If, at the same time, the extra-pancreatic actions of the extract increase the need for insulin greatly in relation to the functional capacity of the beta cells, then degeneration would occur in the islets

and this might overshadow the proliferative effects. If, however, the extra-pancreatic actions were slight in relation to the functional capacity of the beta cells, then the new formation of beta cells would result in an increase in islet volume and insulin content. It has been suggested (9) (74) that if the extra-pancreatic effects of pituitary extracts could be reduced while the proliferative effects were still obtained, then an increase in islet tissue and more nearly normal islet function might result. As we shall see in the discussion of the effects of insulin, the administration of insulin along with pituitary extracts tends to prevent the degenerative changes in the islets while allowing proliferation to occur (10) (74). Extracts of anterior pituitary glands which are pancreatrophic but not diabetogenic usually do not improve the diabetes in the *permanently* diabetic dog (135a). However, these animals may be incapable of forming new beta cells (115) (9). It would be important to see if animals found to be responsive to the diabetogenic pituitary extracts could be made resistant to these extracts by pretreatment with pancreatrophic pituitary preparations or by the previous simultaneous administration of anterior pituitary extracts and insulin.

The administration of insulin. Experiments involving the administration of insulin help to throw some light on the manner in which fasting, fat-feeding and injections of pituitary extracts bring about their effects on the insulin content of pancreas. Some of the effects concerning insulin administration have already been mentioned briefly in the discussions of the effects of fasting, fat-feeding and of anterior pituitary injections. Haist and Best (13) (78) observed that when adequate amounts of protamine zinc insulin are injected into rats receiving the same caloric intake as the controls, the insulin content of the pancreas is reduced. The reduction is less pronounced if the insulin-injected rats eat ad libitum. Various reports indicate that, following extensive insulin administration, changes in carbohydrate tolerance are evident which are similar to those occurring when fat is being fed or the individual is fasting (149) (150) (151) (152) (153) (154). Lacoste, Aubertin and Saric (155) report that injections of insulin twice daily into dogs lead to an increase in the insulin content of the pancreas. The values per kilogram of pancreas which they give are extremely low and their differences do not seem to be significant. These observers also noted an increase in the number and volume of the islets in insulin-treated dogs. Several other observers report hyperplastic changes following repeated doses of insulin in the guinea pig, dog and white rat (156) (157) (158) (159) (160). Schereschewsky and Moguilnitzky (161) found only congestion of the islets after massive doses of insulin. McJunkin and Roberts (162) report that excessive insulin administration in young rats inhibits the proliferative activity of the islet cells although the weight gain of the animals continues to be as great or greater than normal. In the pigeon, large doses of insulin induce atrophy of the beta cells of the islets (163) and, in the rat, depressive changes occur (164). With large doses in the rat, Latta and Harvey (165) found a succession of changes leading to shrinkage of the beta cells, followed by practically complete disappearance of the specific granules of these cells and nuclear changes. After cessation of the injections the beta cells regained their normal granulation and appearance. A

reduction in the specific granules of the beta cells after insulin administration had been commented on previously by Haist and Best (11). Several observers (165) (166) (167) failed to find any evidence of hyperplasia following insulin injections. Mirsky and associates (167a) observed that in a few young, partially depancreatized dogs a persistent diabetic condition could be produced by excessive and prolonged insulin administration. Foglia (168) reports that the pancreases from dogs receiving repeated injections of insulin plus sugar for 37 days, when transplanted into the necks of depancreatized dogs, brought down the blood sugar as quickly as pancreases from normal animals. It should be pointed out that the depressive changes in the islets and in the insulin content of pancreas followed massive doses of insulin, whereas the evidences of hyperplasia usually were reported when smaller doses were used.

The changes in the insulin content of pancreas and in the islets when large doses of insulin are injected, appear to indicate that insulin in excess reduces islet function. Latta and Harvey (165) state that, following repeated injections of insulin the histological changes indicate an almost complete suppression of metabolic activity in beta cells. It seems reasonable to conclude that insulin administration tends to diminish the production and the liberation of insulin by the pancreas. If this is true then the fall in insulin content results from a greater decrease in production than in liberation. With the resultant lower insulin level in the pancreas, however, liberation is reduced also until production and liberation are again balanced at the lower level. Whether the effects of insulin administration are exerted by its influence on blood sugar level or blood insulin level, or indirectly through the pituitary or liver, is not known. Since the effect of fat-feeding on the insulin content of pancreas is similar and may still be obtained in the absence of the pituitary gland, the pituitary mediation of the effect is not likely.

As has already been pointed out, the injection of protamine zinc insulin into starving rats or into rats fed fat, reduces the insulin content of pancreas to a much greater extent than would result from fasting or fat feeding alone (13) (78). On the other hand the administration of insulin relieves the diabetes in partially depancreatized diabetic dogs and cats and restores the islet cells (169) (170). It prevents the great reduction in insulin content and the degranulation and degeneration of beta cells that would otherwise result from the injection of diabetogenic anterior pituitary extracts (10) (124) (125) and in permanently diabetic cats, restores the islets (125). In pituitary-injected dogs receiving insulin the degenerative islet changes were in most instances prevented, but the proliferative ones were still observed (10) (74). It would seem that the pancreatrophic effect of the pituitary extracts was still obtained in these animals, but that the degeneration of beta islet cells did not occur, presumably because the extra-pancreatic effects of the pituitary administration were greatly reduced by the injection of insulin.

Recapitulating then, injections of insulin in adequate amounts have been shown to reduce the insulin content of pancreas; they enhance the insulin-lowering effects of fat-feeding and of fasting in the rat but tend to reduce the effect of

anterior pituitary extract and of partial pancreatectomy on the insulin content of pancreas and histology of the islets in the dog. It seems clear that the effects of fasting, fat-feeding and insulin administration are similar in some respects and opposed to the effect of injections of anterior pituitary extract and of partial pancreatectomy. The evidence supports the belief that the pituitary extracts and partial pancreatectomy lead to an exhaustion of the islet cells through overwork, whereas the administration of insulin, fasting and the feeding of fat, reduce the need for endogenous insulin and decrease the work of the beta cells of the islets of Langerhans.

OTHER TYPES OF EXPERIMENTAL DIABETES. The changes in the insulin content of pancreas in pituitary diabetes have been referred to previously. Three other methods of producing experimental diabetes should be mentioned. These are partial pancreatectomy, the administration of phlorizin and the administration of alloxan.

Partial pancreatectomy. Homans (5) and Allen (6) showed that removal of a large part of the pancreas in dogs led to diabetes associated with degranulation and hydropic degeneration of the beta cells of the islets of Langerhans. Concerning insulin changes in partially depancreatized animals, Haist and Best found that when a portion of the pancreas was removed from dogs and the animals did not become diabetic, the concentration of insulin in the remnant remained within the normal range, but when sufficient pancreas was removed so that the animals did become diabetic, then the insulin concentration in the pancreatic remnant fell to very low values (13) (14). Allen (6) attributed the islet changes to exhaustion through overwork, a view supported by the insulin studies (14). Proliferative changes in the pancreas rather similar to those resulting from injections of anterior pituitary extracts have been reported to occur in the pancreatic remnants following partial pancreatectomy in the guinea pig (171) (172), dog (172) (173), and rat (174). Whether or not the changes in pancreatic islets in the partially depancreatized dog are due to a pituitary influence cannot be decided at present. In the absence of the pituitary the marked lowering of insulin content in the partially depancreatized dog does not occur, even though the pancreatic remnant is very small (14). Either the pituitary is in some way directly responsible for the effect on the islets or else, in the absence of the pituitary, heavy demands on the pancreas for insulin are not made.

Phlorizin. The administration of phlorizin leads to a state which presents some of the features of diabetes but differs in important respects. Phlorizin administration reduces the reabsorption of sugar in the kidney tubules (175) and hence leads to a greatly increased excretion of sugar in the urine. The blood sugar level is lowered and there is an increased new formation of sugar in the liver (176).

Nash and Benedict (177) found that extracts of pancreas from phlorizinized dogs lowered blood sugar in a normal fashion and Cori (178) reported no appreciable difference between the insulin content of two phlorizinized and two normal cats. These were not particularly accurate assays but indicated that insulin was still present in the pancreases of phlorizinized animals. Houssay and Foglia

(179) found that pancreases from phlorizinized animals when grafted into the necks of depancreatized dogs had a diminished ability to restore the blood sugar to normal. They concluded that the insulin secretion was diminished. Lesions in the beta cells of the islets in phlorizinized animals were found by Porto (180). On the basis of these results one might predict that the insulin content of pancreas in the phlorizinized animals would be reduced. Opposed to this is the report that islet hypertrophy has been noted in the guinea pig after phlorizin administration (181).

Alloxan. Alloxan, first observed to have a hyperglycemic action by Jacobs (182) has been shown by Shaw Dunn and his colleagues to cause necrosis of islet tissue in the pancreases of rabbits (183) (184). With the development of islet lesions as a result of repeated injections of alloxan, rats develop persistent glycosuria, hyperglycemia and other changes characteristic of diabetes (185). Hughes, Ware and Young (186) showed that in the rabbit the hypoglycemic effect of alloxan can be simulated by the injection of the amount of insulin calculated to be present in the normal rabbit pancreas. They suggest that preformed insulin is liberated by necrotic islet cells to give the hypoglycemia. The islet necrosis in animals injected with alloxan would lead one to expect a reduced insulin content of pancreas. Recently, Goldner and Gomori (187) reported that values for the insulin content of the pancreas in 2 alloxan diabetic dogs were very low, and some preliminary results obtained by Ridout and Wrenshall (personal communication) indicate that the insulin content of pancreas in rats is reduced after alloxan administration. In 48 hours the insulin content had fallen to 0.1 unit per rat in the alloxan-treated group as compared to 1.1 units of insulin per rat in the *paired-fed* control group.

Human diabetes and the insulin content of the pancreas. Pollak (52) found that the concentration of insulin in the pancreas of diabetic humans was much less than normal. His normal values, however, were much lower than those reported later. Scott and Fisher (53) found that the insulin content of the pancreas in 14 nondiabetic human patients averaged 1.7 units per gram of pancreas (173 units per pancreas). In 18 diabetics the insulin concentration averaged less than 0.4 unit per gram of pancreas or a total of less than 40 units per pancreas. One moderately severe diabetic patient kept under control with insulin for many years showed a content of 1.9 units per gram or 162 units total insulin in the pancreas; in other words, a normal insulin content. One patient admitted in diabetic coma and not responding to insulin in the 36 hour interval before death, had an insulin content of less than 0.06 unit per gram, or a total of 3 units in the whole pancreas. The high value in the insulin controlled diabetic is in keeping with the results of adequate insulin administration in animals receiving diabetogenic anterior pituitary extracts. The findings in islet studies on the pancreases of human diabetics vary greatly but the general conclusion is that there is no characteristic islet lesion in diabetes. However, as several observers have pointed out, while the islets may be normal in appearance in hematoxylin and eosin sections, specific granule stains might show extensive degranulation of beta cells to be present. The insulin concentration closely parallels beta cell granula-

tion in other studies and one would expect from the insulin assays that beta cell granulation would be reduced. The human material will vary greatly in the time after death at which the pancreas is taken for study and in the conditions under which it is kept. This in itself may lead to changes in insulin content and in the granulation of the beta cells of the islets.

Stimulation of islet secretion. The factors involved in the stimulation of insulin production and secretion by islet cells are not clearly established. Many consider that an elevation of the blood sugar level is the main stimulus for secretion. Evidence in favor of this view was to be found in the observations that injections of sugar or hyperglycemic blood into the artery of a pancreas transplanted to the neck (188) or in its normal location (189) caused a fall in the blood sugar level, whereas the same amount of glucose injected elsewhere gave, if anything, a slight increase in blood sugar.

Allen (190), working with *partially depancreatized* dogs, found that keeping the blood sugar at normal levels or below by the administration of phlorizin did not prevent the development of the degenerative changes in islet cells. He concluded that the stimulus leading to hydropic degeneration was some humoral factor, not the excessively high blood sugar but possibly a *deficiency of its own secretion, insulin,* in the circulating blood. More recently, Lukens, Dohan and Wolcott (191) observed that phlorizin treatment restored the blood glucose to normal levels and prevented the development of islet lesions in pituitary-injected dogs. As a result of their findings they consider it likely that *hyperglycemia* is important in the pathogenesis of diabetes. It is hard to believe that these differences in the effects obtained with phlorizin are due to the differences in the prepations used, but they are difficult to explain on any other basis.

Woerner (192) maintained the blood sugar above the normal level in guinea pigs by the continuous intravenous injection of dextrose and found that there was first a reduction in the granulation of beta cells followed by an extensive increase in islet tissue. Using larger amounts of sugar he found that the majority of the beta cells were exhausted or showed the beginning of degenerative changes (193). Houssay and associates (127), using a continuous intravenous infusion of glucose in dogs, kept the blood sugar for 4 days at levels approximating those obtained after pituitary injections. None of the degenerative lesions were evident; the islets, on the contrary, showed signs of hyperplasia. They concluded that the pituitary action on islet cells was not mediated by hyperglycemia. Gomori, Friedman and Caldwell (194) observed that when the blood sugar was elevated in guinea pigs the specific beta cell granules disappeared and when the blood sugar returned to normal the beta cell granulation was restored.

While the evidence for the influence of blood sugar level on insulin secretion is accepted generally, yet there is some indication that the blood insulin level may be involved. It is impossible to say which of the two is the factor of prime importance or whether both are concerned in islet stimulation. Blood sugar level and blood insulin level probably change at the same time but the rôle of blood insulin level cannot be assessed properly until better methods for measuring

insulin in the blood are available. Even when the blood insulin level can be measured accurately, the problem still will not be simple because the level of insulin in blood will depend not only on the rate of insulin liberation by islet cells but also upon the rate of removal of insulin from the blood.

Some observers ascribe a large part in the regulation of insulin secretion to nervous factors. For a discussion of this aspect of the subject the reader is referred to publications by Houssay (195) (196) and Jensen (197). Houssay and his associates hold that the essential regulation of the secretion of insulin is by humoral factors, but that in the dog the nervous system does play a part, though a secondary and dispensable one.

The rôle of the pituitary in the stimulation of insulin secretion is not definitely established. It would appear that there is a fundamental control of islet activity which is independent of the pituitary gland. This normal control probably is a humoral one, though nervous factors may modify it somewhat. In addition to this fundamental regulation, a second mechanism is suggested by the results of the injections of anterior pituitary extracts. These seem to indicate that under certain experimental conditions a direct pituitary stimulation of the islets may occur. Whether or not this superimposed pituitary stimulation plays any part in the normal animal cannot be decided at present. It might be visualized as a special means for enhancing islet secretion when the need for insulin is greatly increased.

SUMMARY

From the literature reviewed it is evident that insulin is produced in the pancreas by the beta cells of the islets of Langerhans and that the regulation of its level is a matter of some complexity. There are good methods available for its extraction, and the procedures for testing the potency of the extracts are satisfactory though not as precise as could be desired. The distribution of insulin is not uniform throughout the whole pancreas, hence small samples may give erroneous results. The manner in which the results of the assays are expressed is also very important.

The value for the insulin content of the pancreas in normal mammals is remarkably similar in different species, though the age of the animal and the diet taken will affect it. No definite seasonal change in insulin content has been established, but unaccountable fluctuations in level occur. Anesthesia with barbiturates does not greatly alter it. Infection, under some circumstances, has a questionable effect which may be the result of a diminished caloric intake. Ligation of the pancreatic ducts reduces the size of the pancreas and also the total amount of insulin present in that organ. Tumors of the islets usually contain relatively large amounts of insulin which may in some instances approximate the amount calculated to be present in *pure islet* tissue. In human diabetes the insulin content of the pancreas is low. This finding is similar to that obtained in experimental diabetes, for the content is greatly reduced after extensive partial pancreatectomy, administration of alloxan or injections of certain anterior pituitary extracts.

One of the most important factors affecting the insulin content of the pancreas is the diet. The insulin content of pancreas in the rat is reduced by fasting, lessening the caloric intake of a balanced diet and by fat-feeding. The reduction seems to be related to a deficiency of carbohydrate or carbohydrate-forming substances in the diet. Diets rich in carbohydrate, on the other hand, do not appreciably increase the insulin content of the pancreas above the *normal* level. The effect of feeding different diets after the insulin content has been reduced by fasting gives some indication that dietary factors other than carbohydrate may be involved in the regulation of the insulin content of the pancreas. This conclusion is supported by the fact that diets deficient in certain amino acids and not adequate for growth or maintenance occasion an appreciable reduction in insulin content. No specific effects of vitamins or of zinc feeding on the insulin content of pancreas have been established.

The injection of large doses of insulin in the rat leads to a reduction in the insulin content of the pancreas and to depressive changes in the islets. Insulin administration in fasting or fat-fed rats greatly augments the effect of these procedures, giving a still further reduction in insulin content.

The insulin-reducing effect of fat-feeding in rats can still be obtained after removal of the gonads, adrenals or pituitary, and, in the adrenalectomized and hypophysectomized animal, the low content resulting from fat-feeding can be restored by giving a balanced diet. The removal of these organs does not in itself lead to an appreciable change in the insulin content. The findings indicate that normally there is a fundamental regulation of the insulin content of pancreas that does not involve the pituitary, adrenals or gonads.

Injections of certain anterior pituitary preparations, however, lead to an increase in the insulin content of the pancreas in the rat. Oestrogenic materials and thyroxin also have a similar action. Some of the effects appear to be mediated through the pituitary gland. In most instances when the insulin content of the pancreas is elevated there is some evidence that the treatment leads to an increase in islet volume. The increase in insulin content therefore is presumably related to the increase in the volume of functioning beta cells.

The administration of diabetogenic pituitary preparations or extensive partial pancreatectomy in the dog results in a very pronounced lowering of the insulin content of the pancreas accompanied by degranulation and hydropic degeneration of the beta cells of the islets of Langerhans.

It is apparent that the insulin content of the pancreas may be reduced by two different and opposed groups of conditions. One includes fasting, fat-feeding and the administration of insulin in the rat; the other takes in extensive partial pancreatectomy and the injection of diabetogenic anterior pituitary extracts in the dog. The treatment with insulin helps to show up certain differences in the two groups. Insulin injections enhance the effect of fasting and fat-feeding in the rat, reducing the insulin content of pancreas to very low values, whereas insulin tends to prevent the lowering of insulin content or the islet changes that would ordinarily result from pituitary injections and partial pancreatectomy. The fact that fat-feeding and fasting also reduce the effect of these procedures

makes the contrast between the groups more striking. The evidence seems to support the view that fasting, fat-feeting and insulin administration reduce the need for endogenous insulin and lower the insulin content of pancreas by making the islet cells less active. In this group the lowering must result from a diminution in production relative to any change in liberation. On the other hand, the great increase in islet stimulation resulting from the injection of anterior pituitary extracts or partial pancreatectomy probably leads to exhaustion of the beta cells through overwork. The lowering of the insulin content of pancreas in this group would seem to result from an increase in the liberation of insulin that is out of proportion to any change in the production of that material.

The factors primarily responsible for the stimulation of the beta cells of the islets are not clearly established. Some contend that the blood sugar level plays the chief part in this regulation, though others believe that the blood insulin level may be involved. The nervous system has only a minor rôle. While the fundamental control of the insulin content of the pancreas is not dependent upon the pituitary, adrenals or gonads, nevertheless, under certain experimental conditions, it would appear that a pituitary effect may be superimposed upon the essential mechanism. It has not been demonstrated that such a direct pituitary influence operates in the normal animal, but if it does it would provide for an additional increase in the secretion of insulin when the need is great.

The evidence indicates that, in experimental animals, several means are available for reducing islet stimulation and lessening the strain on the beta cells. There is reason to believe that the same procedures are effective also in humans. While it is true that by their use islet damage can be prevented and the beta cells restored, yet, on the other hand, their excessive application may lead to poor nutrition of the animal and, in some instances, to atrophy of the islets. Since excessive "rest" may be undesirable as well as excessive stimulation, discretion must be used in the clinical application of these findings.

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PHYSIOLOGICAL ASPECTS OF HUMAN GENETICS, FIVE HUMAN BLOOD CHARACTERISTICS

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As new facts are uncovered relative to the genetics and physiology of human variations it becomes of interest to attempt to correlate and integrate them. In this paper we have tried to do this for five human blood characteristics. The five we have chosen for consideration are: 1, the M-N blood types; 2, the A-B blood groups; 3, sickle cell anemia; 4, hemophilia, and 5, the Rh blood factor.

The M-N blood types. In 1927 Landsteiner and Levine (72, 73) reported that human bloods fall into three types, depending upon the presence of one or both of two agglutinogens which they called M and N. (These types are distinct from the better known A-B groups.) An individual may possess only agglutinogen M in his red blood cells, only N, or both M and N. No individual has ever been found to be lacking in both.

The M-N blood type of an individual is revealed by testing his blood with anti-M and anti-N sera produced in rabbits or in some other laboratory animal. (For methods of technique, see 148, 118.) Anti-M and anti-N agglutinins are normally not developed in human blood, that is, they are normally not isoagglutinins.

In 1928 Landsteiner and Levine (74) announced that the M-N blood variations are entirely genetically determined and that they are inherited in a relatively simple fashion. According to them the observed M-N types result from variations in a single pair of autosomal alleles. (By a pair of alleles we mean a pair of genes occupying the same locus on a pair of homologous chromosomes. The term autosomal implies that the genes involved are not located on the sex chromosomes but on one of the other twenty-three pairs of human chromosomes or autosomes as they are called.) According to the hypothesis suggested an individual develops only agglutinogen M when he is homozygous for one of the alleles, only N when he is homozygous for the other one, and both M and N when he is heterozygous. Many later studies (see 148 and 11) have substantiated fully these conclusions.

When it has been definitely established that a set of alleles is involved in the determination of variations within a character it is conventional and extremely desirable to assign a common symbol to the locus of the alleles. Generally an abbreviation of the character in question is chosen. The different alleles are distinguished by capitalization or by sub- or superscript. Landsteiner and Levine have never assigned any genetically appropriate symbols to the two alleles concerned in the determination of the M-N blood types. In 1941 Strandskov (130) suggested A^m and A^n. (A was chosen as an abbreviation of the term agglutinogen.) If we adopt these symbols the genotypes of the three M-N blood types are:

BLOOD TYPE OR PHENOTYPE	GENOTYPE
M	A^mA^m
MN	A^mA^n
N	A^nA^n

On the basis of the information given above it can easily be shown that the offspring results expected from the various M-N matings are:

MATING	OFFSPRING RESULTS EXPECTED	
	Genotypic ratio	Phenotypic ratio
1. $A^mA^m \times A^mA^m$	*1 A^mA^m	1 M
2. $A^mA^m \times A^mA^n$	$\frac{1}{2}$ A^mA^m: $\frac{1}{2}$ A^mA^n	$\frac{1}{2}$ M: $\frac{1}{2}$ MN
3. $A^mA^m \times A^nA^n$	1 A^mA^n	1 MN
4. $A^mA^n \times A^mA^n$	$\frac{1}{4}$ A^mA^m: $\frac{2}{4}$ A^mA^n: $\frac{1}{4}$ A^nA^n	$\frac{1}{4}$ M: $\frac{2}{4}$ MN: $\frac{1}{4}$ N
5. $A^mA^n \times A^nA^n$	$\frac{1}{2}$ A^mA^n: $\frac{1}{2}$ A^nA^n	$\frac{1}{2}$ MN: $\frac{1}{2}$ N
6. $A^nA^n \times A^nA^n$	1 A^nA^n	1 N

* The coefficient 1 implies unity or all.

The expected mating results presented above are obtained by applying the compound probability law of independent events. For the calculation of expected results the form used in algebra is the most suitable.

Example:

Mating ♀ MN x ♂ MN or ♀ A^mA^n x ♂ A^mA^n
eggs expected = $\frac{1}{2}$ A^m: $\frac{1}{2}$ A^n
sperms " = $\frac{1}{2}$ A^m: $\frac{1}{2}$ A^n

$\frac{1}{4}$ A^mA^m: $\frac{1}{4}$ A^mA^n
$\frac{1}{4}$ A^mA^n: $\frac{1}{4}$ A^nA^n

Genotypic ratio expected = $\frac{1}{4}$ A^mA^m: $\frac{2}{4}$ A^mA^n: $\frac{1}{4}$ A^nA^n
Phenotypic ratio expected = $\frac{1}{4}$ M: $\frac{2}{4}$ MN: $\frac{1}{4}$ N

The A^m and A^n alleles have been shown to be inherited independently of the alleles which are responsible for the A-B blood group variations (6, 145).

According to the universally accepted tenets of genetics every cell of the body of a given individual possesses the same gene complex as did the zygote from which the individual developed. Hence, for example, every cell of an MN individual possesses an A^m and an A^n gene. From all indications, however, M and N agglutinogens are produced only within red blood cells. At least Boyd and Boyd (12) and Wiener and Forer (149) were unable to detect M and N agglutinogens in tissues other than blood. Boyd (10) even tested spermatozoa. Hence it seems probable that only in red blood cells are environmental conditions favorable for the action of the A^m and A^n genes, at least in so far as the production of M and N agglutinogens is concerned. The possibility exists that the A^m and A^n genes are responsible for the catalysis of physiological processes in other cells which produce other types of variations in those tissues, but so far no such

effects have been reported. For a definition of a gene and a general discussion of gene physiology, see Wright (161).

We have already pointed out that every red blood cell of the heterozygote (A^mA^n) possesses both M and N agglutinogens. This must mean that the two alleles A^m and A^n are responsible for the catalysis of somewhat independent chemical processes. This is a rather unusual condition. Two alleles in a heterozygote which is distinguishable from either homozygote generally produce an intermediate effect in one characteristic rather than two separate and clearly distinguishable characteristics such as the M and N agglutinogens.

Although both the M and the N agglutinogens are present in the heterozygote neither is as strongly developed in it as it is in its respective homozygote (73, 74, 154). This could mean that a single dose of each allele can not effect the catalysis of chemical processes to the same extent as two of them can when together. Another possible explanation is that in the heterozygote both the A^m and the A^n alleles draw upon the same substrate and that there is an insufficiency of this material for a complete expression of each gene.

Since the M and N agglutinogens are found only within red blood it seems probable that the effects of the A^m and A^n genes are entirely intracellular. This is in contrast to many other human genes which produce extracellular effects through hormone systems or other cell products. In turn the physiological activities of the M-N alleles apparently are not influenced by hormone systems, at least not sex hormones, because we find the same M-N phenotypic frequency among the two sexes.

The A^m and A^n genes apparently are fully active prior to birth. Moureau (98) has demonstrated the existence of M and N agglutinogens in the blood of human embryos as early as the second month of pre-natal life. Hyman (66) found no changes in type following birth. She believes that the type is fully established by the seventh month of intra-uterine development if not earlier.

It is not only possible to study the genetics of an individual but of a population as well. This analysis consists in part, at least, of determining the relative frequencies of the alleles of every known gene locus. When a character is inherited in as simple a fashion as are the M-N blood types it is actually possible to count the number of each allele within the population. For example, if it were found as a result of tests, that a population in equilibrium consisted of 36,000 M, 48,000 MN and 16,000 N individuals, the relative frequencies of the A^m and A^n genes would be 60 per cent and 40 per cent respectively. For any population the percentage frequency of the two M-N alleles are:

$$A^m = M + \frac{MN}{2}$$

$$A^n = N + \frac{MN}{2}$$

It is, of course, often impractical to test all individuals in a given population with respect to variations in an inherited character but sufficiently large samples for statistically reliable estimates are generally not too difficult to obtain.

The frequencies of A^m and A^n genes have been determined for a large number

of populations. Nearly every racial group has been examined. It is not our intention to give here a complete list of such frequencies. We shall present only the frequencies of three populations in table 1 for illustrative purposes. (For extensive tables see 148, 11, 135.)

When gene frequencies with respect to a particular character have been determined for a given population it becomes of interest to attempt to account for them. Presumably in the evolutionary history of man one member of each set of his alleles was the parental gene and gave rise by mutation to the other allele (or others). As regards the A^m and A^n alleles there is little evidence as to which came first. Agglutinogens serologically similar to M and N have been found in the chimpanzee (73, 148, 23). Hence it seems probable that the origin of both the A^m and the A^n gene antedate man's origin. Agglutinogens similar to M, but none similar to N, have been reported for orang-utans, gibbons and old world monkeys (22, 144). This suggests that the gene A^m may be the older and the parent of A^n, but the evidence is not conclusive.

TABLE 1

POPULATION	INVESTIGATOR	NUMBER TESTED	PERCENTAGE FREQUENCY OF PHENOTYPES			PERCENTAGE FREQUENCY OF GENES	
			M	MN	N	A^m	A^n
U. S. whites, N. Y. City	Landsteiner and Levine	532	26.1	53.6	20.3	52.9	47.1
U. S. negroes, N. Y. City	Landsteiner and Levine	181	27.6	47.5	24.9	51.35	48.65
American Indians from Lawrence, Kansas	Landsteiner and Levine	124	58.1	36.3	5.6	76.25	23.75

Even though we accept the suggestion that one of the M-N alleles has arisen by mutation from the other we have not accounted for the fact that A^m and A^n genes are nearly equally common in most human populations, nor have we accounted for the slightly different frequencies between different populations. It must be obvious that if a single mutant gene appears in a large population it will immediately have only a very low frequency. How then can it increase in proportion? There are three major ways in which this can happen. These are: *a*, recurrent mutation; *b*, accidents of sampling, and *c*, selection. There is no evidence that recurrent mutations have occurred or are occurring within the M-N allelic set but this process could be going on at a fairly high rate without detection, because unexpected variations within M-N blood types are not easily recognized. (Recurrent mutations in other human allelic sets have been reported (52, 53).) Of course, if only one of the alleles mutated to the other the first would eventually become extinct. The present day observed A^m and A^n frequencies could represent either an intermediate stage in a one way mutation system or an equilibrium or an approach toward an equilibrium in a system involving mutations in both directions. (For a detailed discussion on theoretical

consequences of mutations and mutation rates in evolutionary systems see 157, 158, 159, 38, 51.)

If a population is small, accidents not related to survival value may accumulate and shift gene frequencies considerably (see 157, 159, 160, 162). Theoretically the shift should of course occur in both directions but conditions might be altered so that a change in one direction persisted. It is true that most present day intrabreeding human populations are large, but it is not improbable that the primate population which diverged in the direction of man was small and therefore provided an opportunity for an accidental increase in one or the other of the M-N alleles. The observed differences in A^m and A^n gene frequencies between different present day human populations could possibly be explained entirely in terms of accidental shifts.

Selection obviously may change gene frequencies in a population. There is, however, no evidence that a differential survival value exists for M-N alleles. In fact, there is no evidence that the agglutinogens M and N serve any function whatsoever in the human system. Of course, the possibility that the A^m and A^n genes produce other effects which have a differential survival value must be allowed. (For a detailed discussion on the consequences of different selection pressures see 157, 158, 159, 51, 38, 160, 162.)

Since isoagglutinins for the M-N agglutinogens are normally not developed within human individuals no consideration need be given to M-N blood types in blood transfusions. A knowledge of the genetics of M-N blood types is, however, of considerable value in legal cases involving disputed parentage. Tests can not prove that a given individual is the parent of a given child but they may prove that a given individual is not the parent. A further value of a knowledge of the genetics of M-N blood types in populations is to be found in its application to the diagnosis of twins and in problems relating to racial interrelationships.

The A-B blood groups. The A-B blood groups were discovered in 1901 by Landsteiner (69). As pointed out by him there are involved in these blood differences two isoagglutinogens A and B which are located in the red blood cells, and two corresponding isoagglutinins a and b to be found in the blood plasma. A given individual may possess both, only one, or none of the isoagglutinogens. If he has developed a given isoagglutinogen he lacks the corresponding isoagglutinin. On the other hand if he lacks a given isoagglutinogen he possesses the corresponding isoagglutinin.

The four A-B blood groups are properly referred to as the Ab, A, B and O groups. For some time these groups were designated by Roman numerals, but it so happened that two different sets of numbers were given them (the Moss and Jansky classification). This situation led to much confusion. To clear up the dilemma the Health Committee of the League of Nations recommended for adoption the letter terminology originally suggested by von Dungern and Hirszfeld and mentioned above. This is known as the International Nomenclature and is now the only system used in scientific publications.

As is well known the four major A-B blood groups have been shown to have a hereditary basis. Epstein and Ottenberg (37) were the first to find evidence of

this. In 1910 von Dungern and Hirszfeld (33) concluded that two pairs of alleles were involved in their inheritance. This two factor hypothesis, as it is called, was generally considered a correct genetic explanation until 1924. It gave expectations which agreed fairly closely with the observed data. In 1924–25 Bernstein (4, 5) pointed out that three autosomal alleles in a population (that is three genes occupying a given locus on one of the autosomes) could also give four phenotypes corresponding to the four A-B groups. Bernstein examined the results of many matings and found that they conformed closely with results expected on the basis of his triple allelomorph hypothesis. In 1931 Strandskov (129) tested the two proposed hypotheses by the Chi Square method and found that the then available extensive blood group data agreed much more closely with the results expected on the basis of Bernstein's hypothesis than with results expected on the basis of the previously suggested two factor one. Many other studies (see 127, 11, 148) have supported Bernstein. Consequently his theory is now universally accepted with modifications as pointed out below.

TABLE 2

BLOOD GROUP	ISOAGGLUTINOGENS	ISOAGGLUTININS	GENOTYPES
AB	A B	— —	I^AI^B
A	A —	— b	I^AI^A, I^Ai
B	— B	a —	I^BI^B, I^Bi
O	— —	a b	ii

Bernstein never assigned appropriate gene symbols to the three alleles which he considered to be involved. In his 1931 paper Strandskov suggested I^A, I^B, and i. (The letter I was chosen as an abbreviation of the term isoagglutinogen.)

In table 2 are shown the relationships of the isoagglutinogens and isoagglutinins within the four A-B blood groups; also shown are the genotypes according to Bernstein's triple allelomorph hypothesis.

Although it is still correct to speak of four major A-B blood groups there is now conclusive evidence that at least one of the groups must be subdivided into two or more subgroups. As early as 1910 von Dungern and Hirszfeld (33) found that when serum from certain group B individuals is mixed with certain group A bloods, until it loses the power to agglutinate the red cells of these A bloods, it still possesses the ability to agglutinate the red cells of other A bloods. This suggested to them that there exist two kinds of A isoagglutinogens and two kinds of anti-A isoagglutinins. That this is true has been substantiated by many later investigations. In 1930 Landsteiner and Levine (77) designated the two A isoagglutinogens as A_1 and A_2. Accordingly group A is now subdivided into subgroup A_1 and A_2 and group AB into A_1B and A_2B.

Although two anti-A isoagglutinins apparently exist in both B and O bloods it has been found that they do not bear an exact one to one relationship with the A_1 and A_2 isoagglutinogens as might be expected. It is true that one of the two existing isoagglutinins reacts mainly with A_1 bloods. It has therefore been

termed a_1. The other, however, does not agglutinate only A_2 blood but reacts about equally well with A_1. It has therefore been termed the common anti-A isoagglutinin and has been designated as a without a subscript. (A more appropriate symbol would seem to be a_{1-2}.)

A third subgroup of A has been reported independently by Fischer and Hahn (39) and by Friedenreich (42, 41) but it apparently is relatively rare and difficult to detect. Subgroups of B have also been reported (91) but they also remain to be generally known.

With the discovery of the A_1 and A_2 subgroups the question arose as to whether they also had a genetic basis. In 1927 Landsteiner and Levine presented evidence that they do. In 1930 Thomsen, Friedenreich and Worsaae (137, 138) pointed out that their inheritance could be explained by assuming a fourth allele in the series suggested originally by Bernstein. That such a fourth allele exists has now been fully established (148, 11). Hence we now recognize alleles I^{A_1}, I^{A_2}, I^B and i. Additional alleles have been suggested for the other sub-

TABLE 3

BLOOD GROUP OR PHENOTYPE	ISOAGGLUTINOGENS	ISOAGGLUTININS	GENOTYPE
A_1B	A_1 B	— — —	$I^{A_1}I^B$
A_2B	A_2 B	— a_1 —*	$I^{A_2}I^B$
A_1	A_1 —	— — b	$I^{A_1}I^{A_1}$, $I^{A_1}I^{A_2}$, $I^{A_1}i$
A_2	A_2 —	— — b	$I^{A_2}I^{A_2}$, $I^{A_2}i$
B	— B	a, a_1, —†	I^BI^B, I^Bi
O	none	a, a_1, b	ii

* Isoagglutinin a_1 is only rarely found in A_2B bloods.

† Both a and a_1 are present in all B and O bloods but their titers vary considerably.

groups which have been observed but since they do not appear to be clearly established as yet, we may omit a discussion of them here.

The relationships of the isoagglutinogens and isoagglutinins within the four major groups and their subgroups are shown in table 3: also shown are the genotypes of each group.

The relationships of the various genotypes to the six definitely established blood groups deserve some discussion. It may be seen that both the A_1 and B isoagglutinogens are formed in the red blood cells of the heterozygote $I^{A_1}I^B$. This must mean that neither of these two alleles is dominant over the other and also that they catalyze distinctly different chemical reactions within the same cells. The same relationships hold for the I^{A_2} and I^B alleles. As we pointed out in connection with the genes responsible for the production of the M-N agglutinogens it is rather unusual for two alleles to produce two distinctly different characteristics in the heterozygote. The I^{A_1}, I^{A_2} and I^B alleles, as might be expected, produce only their respective isoagglutinogens when present in the homozygous condition. According to Thomsen, Friedenreich and Worsaae (137, 138) the I^{A_1} allele is completely dominant over the I^{A_2} allele so that only

isoagglutinogen A_1 is produced in the heterozygote $I^{A_1}I^{A_2}$. The gene I^{A_1} apparently is also completely dominant over i. This last statement has physiological meaning because the gene i has been shown to produce an isoagglutinogen when homozygous. At least an isoagglutinin (anti O) has been found in some A_1 and A_1B bloods which agglutinates all group O bloods (76, 136). The I^{A_2} and I^B alleles are probably not completely dominant over i because some A_2 and some B bloods give slight reactions with anti-O sera. These presumably are those of the heterozygotes $I^{A_2}i$ and I^Bi (136).

Little or no information is available relative to the question of whether a single dose of each of the I^{A_1}, I^{A_2} and I^B genes produces as much isoagglutinogen as do two of them in the homozygous condition.

A-B alleles apparently are functional within nearly all cells of the body. At least A-B group specific substances have been found in most body tissues (71, 148, 11). An exception is the fetal part of the placenta (114, 101). Their absence in that organ may be an evolutionary adaptation which prevented reactions between fetus and mother. Schiff and Weiler (121) have postulated an enzyme in the placenta which destroys the A-B substances which they assume to be produced there. A-B substances have also been found in most body fluids and gland secretions (82, 111, 148, 118). They are abundantly present in saliva and gastric juice.

An interesting variation has been found with respect to the presence or absence of A and B group specific substances in the saliva and other secretions. In some individuals these substances may be present in high concentrations (secretors), whereas in others (non-secretors) they are absent or nearly absent (82, 111, 148, 118). Schiff and Sasaki (119, 120) were able to demonstrate that these variations have a hereditary basis and that they are dependent upon variations at a single autosomal locus. The allele for the ability to secrete (S) is dominant over that for non-secretion (s). The secretor alleles are inherited independently of those for the production of A-B isoagglutinogens.

So far we have not considered the causes of the production of the specific isoagglutinins which are present in the various A-B blood groups. There appear to be several possible but unsubstantiated explanations for their existence. One is that the same alleles which are responsible for the formation of the isoagglutinogens are also responsible for the isoagglutinins (44). This is a rather attractive hypothesis because of the consistency between the A-B genotypes and the isoagglutinin formed. Difficulties, however, arise when one attempts to consider the physiological relationships involved. What reason would there be for a given gene to produce a particular antigen and also a set of antibodies for antigens produced by other genes belonging to its allelic series? That a given gene will not produce an antibody for the particular antigen it develops is logical, but why should it be responsible for the development of antibodies for other antigens within the same species? A second possible explanation (117) is that the A-B alleles are responsible only for the A-B isoagglutinogens but that these in turn initiate the formation of the isoagglutinins. It is possible to imagine that one allele produces small quantities of one isoagglutinogen, whereas

another produces large quantities of it. Furthermore one can imagine that when small quantities of an isoagglutinogen are formed the corresponding antibodies destroy them, whereas when large quantities of an isoagglutinogen are formed the corresponding antibodies are absorbed. Although this hypothesis has certain attractive features it does not fit in well with Bernstein's triple allelomorph hypothesis. It will be recalled that according to this hypothesis individuals belonging to blood group O are homozygous ii and should therefore produce only one kind of antigen: These individuals, however, develop three kinds of antibodies, namely, the common anti-A agglutinin, anti-A_2, and anti-B. A third possible hypothesis is that the A-B alleles are responsible only for the formation of A-B isoagglutinogens and that the corresponding isoagglutinins are formed as a result of other genetic factors. This hypothesis assumes that all the A-B isoagglutinins are formed in all human individuals but that in the presence of a particular antigen the corresponding antibody is absorbed. Bernstein has favored this hypothesis and to the author also it seems the most probable. A possible variant is that each gene which is responsible for the formation of a particular antigen inhibits at the same time the formation of the corresponding antibody. An allele which produced such an inhibition effect would have been strongly selected for in the course of evolution.

If we accept the assumption of this last mentioned hypothesis, namely, that the A-B isoagglutinins are produced normally, i.e., due to other genetic factors, we must account for the existence of such genetic factors. One possible explanation is that the A-B isoagglutinogens are similar to other antigens which are commonly found in lower forms which parasitized man's ancestors. There is some evidence that A-B group specific substances are similar to such commonly found antigens. For example, it has been shown that agglutinogen A resembles the Forsman antigen which is found in many bacteria as well as in many other parasitic forms (70, 27, 35, 116, 43).

Human populations have also been studied with respect to the distribution of the A-B alleles. The formulae for the determination of the A-B gene frequencies from observed phenotypic frequencies are somewhat more complicated than those for the M-N alleles, but they are not difficult to apply. If we assume random mating in a population (and there is every reason to believe that we may) these formulae for the frequencies of the four alleles are obtained from the square of the frequency array of the four alleles ($I^{A_1} + I^{A_2} + I^B, + i$) or the square of ($p_1 + p_2 + q + r$). By taking into account the genotypic and phenotypic relationships, the frequencies of the four alleles in any population, based on the empirically determined A-B phenotypic frequencies are as follows:

$$p_1 = \sqrt{A_1 + A_2 + O} - \sqrt{A_2 + O}$$
$$p_2 = \sqrt{A_2 + O} - \sqrt{O}$$
$$q = \sqrt{B + O} - \sqrt{O}$$
$$r = \sqrt{O} \text{ or } 1 - (p_1 + p_2 + q)$$

Since the fourth allele of the A-B series has been recognized only a relatively short time not many population studies have included it, but the frequencies

with respect to the three originally recognized alleles have been determined for many populations (11, 127). A few sample frequencies including all four alleles are given in table 4.

As suggested by table 4 the A-B gene frequencies vary considerably among different human populations. All four alleles are, however, represented in all or at least in nearly all populations. This suggests that all four alleles were present in the original human stock. This point of view is supported by the fact that agglutinogens serologically similar to the human A-B antigens have been found among Anthropoid apes and lower primate groups (34, 78, 3, 6). There is little or no evidence as to which of the four alleles appeared first in the course of evolution and there is little or no evidence on the basis of which to account for the present day A-B gene frequencies. Mutations within the A-B allelic series have not been reported but again this is not surprising in view of the fact that such mutations are extremely difficult to detect. Selection may have played a rôle in decreasing or increasing a given gene's frequency within a

TABLE 4

POPULATION	INVESTIGATOR	NUMBER TESTED	PERCENTAGE FREQUENCY OF GROUPS						PERCENTAGE FREQUENCY OF GENES			
			A_1B	A_2B	A_1	A_2	B	O	I^{A_1}	I^{A_2}	I^B	i
U. S. whites	Wiener and Sonn	1077	5.2	1.4	29.0	8.9	13.9	41.7	18.1	6.5	10.0	64.6
U. S. negroes	Wiener	189	1.6	1.1	19.6	6.8	22.8	48.1	12.2	4.8	14.5	69.4
Full-blooded American Indians	Landsteiner, Wiener and Matson	120	0	0	25.8	0	0.8	73.3	13.9	0.0	0.5	85.6

particular population but actually there is no conclusive evidence that any of the A-B alleles have a greater or a lesser survival value than any of the others. Accidents of sampling could account for some of the frequency differences between populations, particularly differences between small populations such are found among some of the North American Indian tribes.

In contrast to the M-N blood types the A-B blood groups must be considered in blood transfusions. Some consider group AB individuals universal recipients and group O individuals universal donors. It is, however, advisable to use, except in emergencies, only donors of the same blood group as the host. In addition to their importance in blood transfusions A-B blood group determinations are exceedingly valuable in the solution of cases of disputed parentage. However, as was true for the M-N blood types no individual on the basis of A-B determinations can be proven to be the parent of a given child. He may only be shown not to be the parent. A-B blood group determinations have also wide application in the diagnosis of twins and in problems relating to racial origins.

Sickle cell anemia. Sickle cell anemia was first reported by Herrick (61) in 1910. The patient was a negro youth from the West Indies. Since this first report many similar cases have been found. The diagnostic feature is the presence of crescentic or sickle shaped red blood cells. These may not be common

in the blood smear but susceptible cells can be induced to sickle by sealing them under a cover slip with petrolatum and incubating them at room temperature for twenty four hours (70) or by a number of other techniques (65, 50, 132, 2, 32).

In 1923 Huck (65) and Taliaferro and Huck (134) presented evidence that sickle cell anemia is a familial disease and that it is inherited as an autosomal dominant. According to this hypothesis all affected individuals possess at least one dominant gene (Si) and all normal individuals are homozygous recessive (si si). Although only a limited number of studies have been carried out to test this mode of inheritance, the combined genetic evidence obtained since then (49, 96, 99, 115) seems to substantiate it.

As we mentioned above, the most striking physiological effect of the Si gene is the production of a condition within red blood cells which makes them susceptible to sickling. What this condition is no one has discovered as yet. There seems no question but what the effect resides within the red blood cells because Huck (65) and Sydenstricker (132) have shown that the red cells of sickle cell patients sickle when washed with serum from normal persons, whereas the serum from the former does not cause cells of the latter to sickle. Emmel (36) has suggested that the sickling may be only an accentuation of the normal process which causes red blood cells to assume the biconcave disk shape.

According to Sydenstricker (132) and Emmel (36) sickled cells are immediately subject to phagocytosis. Large mononuclear cells occur in the blood of sickle cell patients which phagocytose sickled cells but will not attack the red cells of normal blood. Wollstein and Kreedel (141) report that the Kupfer cells are active in the removal of sickled cells. Cardozo (17) has tested for the presence of specific agglutinogens but has failed to find such substances. Hahn and Gillespie (49) have reported that susceptible cells can be induced to sickle in an atmosphere of carbon dioxide and caused to revert to normal shape when saturated with oxygen. However, Hein, McCalla and Thorne (60) and Graham and McCarty (45) did not find increased sickling upon placing susceptible cells in closed chambers.

The Si gene apparently produces its effect early in the development of red blood cells. Cooley and Lee (19) have reported sickled cells that were nucleated and Jaffe (67) and others have observed reticulated cells.

Whether all the red cells of all sickle cell patients are subject to sickling is not certain. Variations occur in the number which respond. Anderson and Ware (1) generalize by saying: "Ninety per cent of the supply of new cells are sickle cells." This, however, does not necessarily mean that the rest are not susceptible.

Other conditions which have been reported to be associated with sickling may be secondary effects of the Si gene rather than primary ones. Anderson and Ware (1) outline secondary effects as follows:

> When they (the red cells of sickle cell patients) are put into circulation all except about 2% of the sickle cells are destroyed by phagocytosis. This brings about a greater activity of the bone marrow in the production of new cells, as is indicated by the increase in reticulocytes. The spleen and liver enlarge to take care of the influx of young cells and a vicious

cycle is established. As fast as the red cells form they become sickle cells and are phagocytosed. The bone marrow cannot continue its hyperactivity indefinitely, and as the disease progresses, fewer new cells are put into circulation. The stimulus that caused the spleen to enlarge is thus removed and it decreases in size. The liver, however, remains enlarged, possibly because its various other functions make it less responsive to the failure of the haematopoietic system.

Some investigators (95, 139, 13, 29, 47, 59) have reported bone changes, particularly in the skull and long bones. Cardozo (17), however, found no such changes in 17 patients which he studied specifically for such effects. With respect to other associated conditions Anderson and Ware (1) write:

The patient usually gives a history of having been weak and sickly for a number of years and of having previous attacks of weakness, jaundice, fever and abdominal or articular pain. There is usually a history of repeated acute infection of the respiratory tract. The course of such infection is usually longer than that in normal persons.

Recently Wertham, Mitchell and Angrist (143) have reported changes in the central nervous system. They find: "focal and diffuse changes in the nerve cells in cortical and sub-cortical gray structures, and focal areas of demyelination in the spinal cord."

For a long time it was thought that sickle cell anemia was limited to the negroid stock. However, in 1929 Cooley and Lee (20) reported a case in a Greek child which could not reasonably be attributed to race admixture. Since then a number of other instances of sickling have been found in non-negroid populations (125, 115, 18, 108, 140, 46, 96, 99). Most of these cases are from descendants of Southern European peoples. Ogden (99) examined 1,602 unselected, consecutive patients which included 692 negroes and 910 whites. Among the negroes he found 45 who showed the sickling trait, whereas among the 910 whites he found none. Ogden writes in 1943 as follows:

I believe I have a right to my strong conviction that the sickling trait is a condition found in the negro race only and that in all cases in which members of white families have such a trait an admixture of negro blood in the immediate or remote ancestry has taken place. . . . In no case of the sickling trait in a white person reported up to the present time has the possibility of negro blood been excluded.

One must admit the possibility of Ogden's contention, but it does seem unlikely that all cases reported among Caucasoids are to be explained in terms of race admixture.

Estimates of the incidence of sickle cell anemia among negroes range from 4.3 per cent (133) to 9.42 per cent (17). Ogden (99) obtained a frequency of 6.5 per cent among 692 unselected negroes. Cardozo (17) combined all published data and arrived at a frequency of 7.44 per cent. This percentage is based on an examination by different investigators of 11,021 individuals.

A difference in sex incidence has been reported which may be significant. Cardozo (17) finds that 69.1 per cent of those showing sickling are females. Ogden (99) in his series found 37 females to 8 males. If this sex difference is a real one there has yet been no explanation advanced to account for it. An

autosomal dominant characteristic should occur with equal frequency among females and males.

It is of interest to estimate the incidence of the Si and si genes in populations. If a trait due to an autosomal dominant gene such as Si has a frequency of 7.44 per cent in a population in which mating is occurring at random the recessive character (in this case the normal condition) obviously has an incidence of 92.56 per cent. The best estimate of an autosomal recessive gene in a population mating at random is obtained by extracting the square root of the incidence of the recessive character in the population. Therefore, it may be estimated that the si gene in negroid populations has a frequency equal to square root of 92.56 or 96.2 per cent. It follows, of course, if only two alleles exist that the dominant gene has an incidence of 3.8 per cent. What the frequency of the Si gene is in Caucasoid and Mongoloid populations can not be determined until more data are available. Perhaps as Ogden insists it does not occur at all in these groups except as the result of race admixture.

As must be obvious from the symptoms associated with sickle cell anemia selection against the Si gene must be fairly strong. Anderson and Ware (1) write: "Owing to his decreased resistance he (the patient) is a prey to the various infectious diseases and usually succumbs to one of these. Few patients live beyond the age of 35 years." If selection operates as strongly as this statement suggests it becomes a problem to account for the Si gene's high incidence among negroes. One possible explanation is that the si gene mutates to the Si gene at a high rate within this group. There is, however, no direct evidence that such mutations are taking place, but it seems almost inevitable that they must be. The lower incidence of the Si gene among Mongoloids and Causasoids could be accounted for either in terms of a lower mutation rate within these groups or in terms of a higher selection pressure.

A knowledge of the genetics of sickle cell anemia has only a limited application to forensic medicine but such knowledge does contribute to our prediction of the occurrence of sickle cell anemia in a family and therefore to our chances of discovering cases in their incipient stages. Studies on the frequency of the Si and si alleles in different populations can contribute to the solution of problems relating to racial interrelationships.

Hemophilia. The earliest unmistakable case of hemophilia was reported in the sixteenth century by Albucasis. However, a clear and concise description was first given in 1803 by Dr. John C. Otto of Philadelphia (102). He was also the first to point out that hemophilia has a hereditary basis, a suggestion which has been fully substantiated. Otto did not suggest any specific Mendelian mode of inheritance. This is not surprising in view of the fact that his publication antedated Mendel's original discovery by more than sixty years. Otto did, nevertheless, present the view that only males show the condition and that they inherit it through unaffected females.

When sex-linked inheritance was discovered in the fruit fly, Drosophila melanogaster, about 1910 it became apparent that hemophilia in man was probably inherited as a sex-linked recessive (97). This implies that the hemophilic gene

(h) and its normal allele (H) have their common locus on the X chromosome, and that a single hemophilic gene (h) will produce the condition in the male, whereas in the female two are necessary for its expression. Nearly all the published pedigrees support this mode of inheritance. Haldane (55) has presented evidence of linkage between the locus of the hemophilic gene and that for red-green color blindness which is known to be sex-linked. This is confirmatory evidence for sex-linkage. After Haldane (52) had published evidence that some other genes of man are partially sex-linked, i.e., carried on the region of the X-chromosome where crossing over occurs with the Y-chromosome, Sirks (126) suggested that the hemophilic gene might also belong in this category. However, as Haldane (58) has already pointed out, Sirk's arguments are not very convincing. A few pedigrees, particularly those of Bess Lloyd (88), suggest that the hemophilic gene, although sex-linked, may not always be completely recessive. She reports four females who were heterozygous and gave evidence of being bleeders. She states: "they generally are not as severely affected as the males." Warde (142) also reports a female as hemophilic who probably was only heterozygous. Her father and only son were hemophilics but her mother had no hemophilic ancestry. Foulis and Crawford (40) reported two female bleeders in a hemophilic pedigree. Their fathers were normal but one of the females had two hemophilic sons which is evidence that she at least was heterozygous, i.e., a carrier. Foulis and Crawford admit that their two cases might represent purpura hemorrhagica rather than true hemophilia, but there at least is a suggestion that the effect was due to the hemophilic gene. Although the few pedigrees we have mentioned suggest incomplete recessiveness on the part of the hemophilic gene most heterozygous females show no effect. Hence it still seems justifiable to conclude that the common form of hemophilia is not only sex-linked but completely recessive. The heterozygous females which show a condition similar to hemophilia may possess a variant of the common allele or present a clinical picture similar to hemophilia due to other factors.

If the gene for hemophilia (h) and its normal allele (H) are sex-linked the expected genotypic results of the six possible matings are as follows:

1. ♀ HH x ♂ H(y) = $\frac{1}{2}$ ♀ HH:$\frac{1}{2}$ ♂ H(y)
2. ♀ Hh x ♂ H(y) = $\frac{1}{4}$ ♀ HH:$\frac{1}{4}$ ♀ Hh:$\frac{1}{4}$ ♂ H(y):$\frac{1}{4}$ ♂ h(y)
3. ♀ hh x ♂ H(y) = $\frac{1}{2}$ ♀ Hh :$\frac{1}{2}$ ♂ h(y)
4. ♀ HH x ♂ h(y) = $\frac{1}{2}$ ♀ Hh :$\frac{1}{2}$ ♂ H(y)
5. ♀ Hh x ♂ h(y) = $\frac{1}{4}$ ♀ Hh :$\frac{1}{4}$ ♀ hh:$\frac{1}{4}$ ♂ H(y):$\frac{1}{4}$ ♂ h(y)
6. ♀ hh x ♂ h(y) = $\frac{1}{2}$ ♀ hh :$\frac{1}{2}$ ♂ h(y)

The expected results are calculated as shown below. Mating no. 5 is used as an illustration.

Mating ♀ Hh x ♂ h(y)
eggs expected = $\frac{1}{2}$ H:$\frac{1}{2}$ h
sperm expected = $\frac{1}{2}$ h:$\frac{1}{2}$ (y)
Genotypic ratio
expected = $\frac{1}{4}$ ♀ Hh:$\frac{1}{4}$ ♀ hh:$\frac{1}{4}$ ♂ H(y):$\frac{1}{4}$ ♂ h(y)

One of the problems relative to hemophilia that has puzzled many people is

the question of why so few females are hemophilics (if any are), when so many males are afflicted. Why this sex difference in incidence? At least a partial answer to this question is to be found in the manner in which hemophilia is inherited. If a character is inherited as a sex-linked recessive, and is relatively rare in a population which is mating at random, it should theoretically be expected to be much more common among males than females. Let us assume for instance that a pair of sex-linked alleles, B and b, have frequencies in a population of 90 per cent and 10 per cent, respectively. Since only a single gene is necessary to express the character in a male these should show the gene frequency directly, i.e., 90 per cent of the males should show the dominant sex-linked character due to (B) and 10 per cent should show the recessive due to b. On the other hand in the female two recessive genes (bb) must come together to show the recessive character. Hence the frequency of a sex-linked recessive character expected among females is only the square of the recessive gene frequency or in the above case 1 per cent. (The probability of two independent events occurring in combination is the product of their independent chances.) The expected difference in sex incidence can be made a little more striking by resort to another example. If for instance a sex-linked recessive character has an incidence of 1 in 100,000 among males we should theoretically expect only 1 female among 10,000,000,000 to show it. The incidence of hemophilia among males is not as low as 1 in 100,000 but it is low enough to give a decidedly large expected sex difference. What the exact incidence of hemophilia is in most human populations has not been determined with any degree of accuracy. Haldane (55) has estimated for the population of London an incidence of about 1 in 10,000 among males. If we accept this figure for every population we should expect only 1 female among every 100,000,000 to be homozygous hh and therefore hemophilic.

Other factors make the occurrence of hemophilia among females even more improbable. In order to produce a homozygous female (hh) it is necessary that her father be a hemophilic and her mother homozygous (hh) or a carrier (Hh). In the latter type of mating a homozygous female (hh) has only a probability of 50 per cent. Now it is well known that hemophilic males tend to die early. Estimates have indicated that 54 per cent of hemophilic males die before the fifth year and that 88 per cent die before the twentieth. Snyder (128) states that only 11 per cent of hemophilic males live to be 22. Thus there is a strong chance that hemophilic males will not marry because of death. Furthermore of those who live relatively few marry. Snyder (128) examined 250 of the published pedigrees on hemophilia and found only one instance where a known hemophilic male had married a known carrier female and where a hemophilic daughter might therefore be expected. This was pedigree no. 407 in the collection published in 1911 by Bullock and Fildes (16). This mating produced one hemophilic son and one normal daughter. Thus from this mating a homozygous female (hh) was probably not even realized.

Some authors have postulated that the hemophilic gene produces a lethal effect early in life when it is present in a double dose in the female. Others such as Birch (9) have suggested that the homozygous female (hh) survives but fails to

show the hemophilic condition due to alleviating effects produced by female hormones. Although these supplementary hypotheses may be correct and may explain in part the complete or nearly complete absence of hemophilia among females they do not seem to be necessary. The probability of a homozygous (hh) female in a given population is practically nil.

Physiologically the hemophilic gene (h) produces interesting and serious effects. The literature on this subject is generally known and has been frequently summarized. It therefore seems unnecessary to review it here. (See 122, 64, 3, 8, 93, 105, 104, 94, 63, 90, 26, 124, 123, 62, 14, 24, 28, 48, 89, 106, 112.)

Hemophilia probably occurs in all racial stocks. Its incidence among Caucasoids, as pointed out above, is not accurately determined for any given population, but estimates have been made for a few of them. Haldane (55) believes that among male births in London the incidence of hemophilia lies somewhere between 35 and 175 per million. Bullock and Fildes (16) think that hemophilia is a good deal more common in Northern than in Southern Europe. They do not give specific data. Komai (68) is of the opinion that the same type of hemophilia which appears in the Caucasoid stock occurs in the Mongoloid. He presents a number of pedigrees which agree with a sex-linked recessive mode of inheritance. He does not give an estimate of its frequency among Mongoloids. Among Negroids hemophilia is practically unknown. Bullock and Fildes (16) found three cases reported among negroes, but they did not consider any of them reliable. In 1936 Crandall (21) reported hemophilia in a full blooded negro and he indicated at that time that he believed his patient to be the only authentic case among negroes. The genealogy according to Crandall did not, however, give evidence of inheritance. In 1937 Pachman (103) reported three cases of hemophilia among negroes which had been seen at Duke Hospital. According to Pachman "Two of them have a definite family history and a genealogy which is fairly typical. In the third case, the diagnosis may be questioned but the history and x-ray findings are typical of hemophilia." He writes further: "the skin and features of cases 1 and 3 were those of a full blooded negro, but in case 2 the color was lighter indicating that a mixture may have occurred."

An interesting problem from a genetic point of view is the question of how the frequency of the hemophilic gene is maintained at as high a level as it is in the face of the selection pressure which it encounters. As mentioned earlier most hemophilic males fail to marry and consequently to leave offspring. Under these conditions alone the (h) gene should gradually be reduced in frequency or eliminated completely. To account for what appears to be a nearly constant frequency for this gene Haldane (52, 55) has suggested that mutations from H to h are taking place. He estimates that one mutation occurs per 50,000 individuals per generation. Enough sporadic cases of hemophilia have been reported to give credence to this hypothesis. No good explanation is available to account for racial differences in incidence.

A knowledge of the mode of inheritance of hemophilia is only of limited value in forensic medicine, but it is of importance to a practising physician in his determination of the probability of its occurrence in a given family. To the human

geneticist hemophilia is an extremely interesting characteristic. It offers unusual opportunities for an analysis of certain aspects of all the various factors which operate in the evolution of man.

The Rh blood factor. In 1940 Landsteiner and Wiener (79) discovered a new blood agglutinogen. It was called Rh because it was revealed as the result of using serum from rabbits into which rhesus monkey blood had been introduced. An interesting discovery with respect to it was that only about 86 per cent of the human white population gave positive reactions (Rh+) to the anti Rh serum while the remaining 14 per cent gave negative reactions (Rh−). The following year the same two investigators (81) tested for the rôle of heredity. They examined 60 families which included 237 children. They concluded that the variations have a hereditary basis and that only a single pair of autosomal alleles is involved. They found that an individual is Rh+ if he possesses at least one dominant gene (Rh) and Rh− when homozygous recessive (rh rh). In a later paper Wiener and Sonn (146) extended this genetic study to include 40 additional families with 138 children. Their results substantiated the previously suggested genetic hypothesis.

In the last year or two evidence has been obtained (146, 150, 84, 83) which suggests that at least 5 different Rh agglutinogens exist. According to Wiener (147) these may occur in different combinations in different individuals and give 8 Rh blood types (including the Rh negative type). The different Rh agglutinogens give different presence and absence frequencies within the same populations. In 1943 Wiener and Landsteiner (150) presented evidence that some of these new Rh subdivisions have a hereditary basis, and that at least three alleles are involved instead of two as was originally suggested. More recently Wiener (147) has postulated 6 alleles. Although Wiener has presented some evidence for the existence of 6 alleles it seems too early to consider all of them fully established.

Although Rh alleles seemingly produce direct effects only within red blood cells, there is considerable evidence to suggest that they may be responsible for rather serious secondary effects. In 1939 Levine and Stetson (87) reported a fatal hemolytic reaction in a woman who had been given blood transfusion following a stillbirth. The transfused blood, according to the usual tests, was compatible with the blood of the host. Yet a reaction occurred. The authors postulated that the fetus had inherited from its father the capacity to develop certain antigens which were not present in the mother and which induced the formation of antibodies in her tissues. Further cases of transfusion reactions which could be attributed to iso-immunization following pregnancy were reported in 1940 by Levine and Katzin (85). The same year Wiener and Peters (151) pointed out that every recorded case of severe hemolytic reactions following first transfusions had occurred in post partum patients. In 1941, following the discovery of the Rh factor and its inheritance, Levine et al. (86) expressed the belief that several cases of miscarriages, stillbirths and of erythroblastosis fetalis which they had encountered were probably attributable to the Rh factor. They postulated that these effects might result when the mother was Rh− and the fetus was Rh+, due to its heredity from the paternal side. They postulated that Rh

antigens of the fetus had entered the maternal blood stream, caused the formation of antibodies in her tissues and that these in turn had filtered through the placenta and reacted with the Rh+ antigen in the fetus. It is of interest that Ottenberg (100) and Darrow (25) had previously suggested a serological explanation for erythroblastosis but had not been able to demonstrate any incompatibility between the blood of the fetus and that of the mother. (For a clinical description and diagnosis of erythroblastosis fetalis see (31, 30, 7, 109, 107, 92, 15).)

That most if not all cases of erythroblastosis fetalis may be attributed to Rh blood factors seems probable. Potter, Davidsohn and Crunden (110) found that among 60 mothers who had given birth to babies which had been diagnosed as erythroblastotic 90 per cent were Rh negative. The infants which had been diagnosed as erythroblastotic and born to Rh+ mothers, these investigators believe, might actually be suffering from "a different disease entity." Another possible explanation is that each of these mothers was negative with respect to an Rh agglutinogen other than the one tested for, whereas the fetus was positive with respect to it.

Although erythroblastosis may result when the mother is Rh− and the fetus is Rh+ it does not follow that it invariably does. The fact is that the disease occurs in only a small percentage of cases where this relationship exists. What the placental peculiarity is which results in the occurrence or absence of erythroblastosis is not known. It is known that if the disease has occurred once in a family it is apt to recur. This may mean that some hereditary basis exists for this placental peculiarity.

Recently Witebsky and Heide (155, 156) have reported the occurrence of Rh antibodies in the breast milk of mothers who are Rh− and whose children are Rh+ and erythroblastotic. These investigators believe that such antibodies might do further damage to the red blood cells of erythroblastotic children who consume such milk.

From the population genetics point of view the Rh factor gives promise of being extremely interesting. As we have already indicated the different Rh agglutinogens have different frequencies within the white population. This means that the various alleles which are responsible for them also have different frequencies within that group. In 1942 Landsteiner, Wiener and Matson (81) reported on the blood of 120 full blooded American Indians and 155 of mixed ancestry. Among the 120 full blooded Indians they found only a single individual who was Rh−. Among the 155 mixed bloods they found an incidence intermediate between that for full blooded Indians and whites. More recently Wiener, Sonn and Belkin (153) have tested Chinese and Negro populations. They found the Chinese like the American Indians to have a very low Rh− frequency. Type Rh, which is a subdivision of the original Rh+ group, and which is rare in the white population, they found to have a low incidence among Chinese but to be very common among negroes.

With the number of Rh alleles unsettled it is too early to discuss in detail the possible rôles of the various evolutionary factors in the determination of Rh

gene frequencies in populations. It is, however, of interest to call attention to the manner in which selection operates. As Haldane (58) has pointed out, selection is against the heterozygote. Therefore an Rh and an rh gene is eliminated every time selection operates. If positive and negative producing Rh alleles were equally common in the population neither of the two types of alleles would be changed in frequency. The negative allele, however, is less common; therefore there is a tendency for it to be decreased in proportion to the positive alleles. The rate at which it is being decreased is, however, a very slow one. Haldane (58) has estimated that it would require 619 generations or about 15,000 years to reduce the frequency of homozygous recessive individuals (rh rh) from its present figure, which is about 14 per cent, down to 1 per cent. This is based on the assumption that no mutations to the rh allele are occurring. There is no evidence that there are. For the rh allele to be eliminated completely, without intervention by man, many more generations would be required.

The question is frequently asked if it would not be desirable to advocate laws to prevent rh rh females from marrying Rh+ males. Although such a law would probably eliminate the occurrence of erythroblastosis fetalis there is no justification for such an extreme measure. The incidence of erythroblastosis from such marriages is not high enough to warrant it. A more reasonable position is taken by Haldane (58). He writes: "Even if no systematic attempt were made to eradicate such a gene there would be a strong case for dissuading rh rh women known to be so constituted as to be destined to form permeable placentae from marrying Rh Rh or Rh rh men." This suggestion seems defensible.

Since iso-immunization to Rh agglutinogens is possible Rh blood types should be considered in blood transfusions, especially if repeated transfusions are to be given.

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VERTEBRATE SMOOTH MUSCLE

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Our present knowledge of smooth muscle is nearly as vague as it was when this subject was last reviewed in this journal. Although 18 years of intensive research have passed in the meantime, the introductory remarks of Evans (81) about the reasons for this lack of precise knowledge could be repeated literally today. The main change in our general understanding of "smooth muscle" is the deepened recognition that the conception of "smooth muscle" as a biological unity is completely misleading. This is true from various points of view. We can regard "striated muscle" of vertebrates as a unity since with negligible exceptions, histological, physiological and pharmacological differences are small, not only between the different anatomical muscles of the same animal but also between muscles from representatives of all five classes of vertebrates. For smooth muscles important differences exist, not only between the various smooth muscle tissues of one animal, but also between anatomically and functionally comparable smooth muscles even of related species. Striated muscles are more or less all organs with the same functions and consist practically of muscle tissue alone. Therefore, they can be easily subdivided into units, motor units, or even single striated fibers, all of which have to a certain extent the same physiological properties as the whole anatomical muscle. As a rule, smooth muscles are only elements participating with various other tissues in forming organs of quite different functions such as stomach, artery, spleen, uterus, iris, etc. If we isolate the muscle elements, either imperfectly in experiments or perfectly in thought, they do not exhibit the functions of the whole organ, but only those connected with contractility. Such isolated smooth muscle elements from various organs are not uniform as to excitability, mediation, inhibition, and conductivity. The contractility they have in common, but they share this with striated muscle and even with other vertebrate tissue elements, such as chromatophores, ciliary epithelium, etc. Thus vertebrate smooth muscle must be defined as: Cells specialized for contraction only with their protoplasmatic constituents homogeneous in the longitudinal direction.

In vertebrates, only in exceptional instances do these smooth muscle cells unite, without important participation of other tissues, to form something like an anatomical muscle: e.g., the pilomotors in mammals, the retractor penis in some species. But in invertebrates, the formation of anatomical muscles from smooth muscle cells is rather common, and since a large part of the experimental work on smooth muscle has been done on invertebrate material, the above outlined difference between vertebrate smooth and striated muscles has often been neglected.

Due to the fact that we have a relatively well established visualization of the physiological properties of striated muscle, it has become customary to express the function of smooth muscles in terms derived from the function of striated

muscles, although often these terms might have little meaning for smooth muscles. But such terms, if used discriminately, help to shorten description and discussion. In a review it is impossible to discuss the properties of the smooth muscle elements separately for each organ and to point out each time the differences between various species. An attempt will be made to show that, on the one hand, smooth muscle tissues of various origins have in common certain properties directly connected with contractility while, on the other hand, there exist important organ and species differences of those properties connected with excitation and conduction.

DOES THERE EXIST AN INTERMEDIATE MUSCLE TYPE? Only during embryological development and in tissue cultures do difficulties arise in classifying muscle cells as smooth or striated. In those exceptional instances in which striated muscle fibers participate with other tissues in forming organs, they have the physiological and the pharmacological properties of true skeletal muscle fibers, i.e., the striated fibers of mammalian esophagi (88, 134), or the striated fibers of the iris of Sauropsidae (33, 132, 164). The latter example is of special interest, since these striated fibers are of ectodermal origin (145), and the dilator at least is smooth in some species of birds (93). A similar variability in the composition of the esophagus musculature is found not only from class to class of the vertebrates (178), but also from species to species in mammals (216) and even between related sub-species (239). But never the least histological or physiological evidence was found for the existence of an intermediate type of muscle cells. In the palatal membrane of many fishes (37, 163), and in some species (i.e., tench) throughout the whole gut (95, 159) striated muscle fibers are found scattered more or less numerously through the smooth muscle coats. Physiologically and pharmacologically, the striated behave like true skeletal muscle, and the smooth ones like gut muscle in general.

STRUCTURE. Despite the diversity of opinion about the nature of the surface of the smooth muscle cells (membrane or only border sheets of hardened sarcoplasm), the histologists agree (107) that a typical membrane comparable to the sarcolemma of the striated muscle does not exist and that in many organs containing smooth muscles, the fibrils run from cell to cell and also form a loose fibrillar net by anastomoses with neighboring cells, thus giving rise to a syncytium-like formation. Roskin (203) points out that in most smooth muscle tissues the smooth muscle cell does not represent a unit and that on the other hand the network of fibrils has not all of the characteristics of a syncytium. He proposes the name "myon" for a single strand of smooth fibrils which can form "myons of higher order" by protoplasmatic anastomoses. There is no doubt that the more superficial fibrils (boundary fibrils) in a cell are coarser than the more central ones. There is a large variation in the number and thickness of the fibrils from one smooth muscle organ to another.

For smooth muscles from the cat intestine two different histological states of contraction have been described (203). In one, the fibrils are straight, while in the other state the fibrils are curled regularly like corkscrews. However, in experiments with the retractor penis and intestinal loops of dogs, it could be

demonstrated that curled fibrils are never seen if the muscles have been fixed histologically after slow contraction against a heavy load. But corkscrew fibrils are found in the inner part of muscles if they shortened quickly before or at fixation (88). These spiral fibrils apparently belong to relaxed myons and are crumpled together by the contraction of adjacent myons.

The submicroscopical structure of vertebrate smooth muscles (retractor penis, gut) as investigated by birefringence measurements revealed marked conformity with that of the A disc of the striated muscle (88). Besides a micellar pattern, there exists a crystalline structure of the micellae themselves. As in striated muscle, the submicroscopical pattern is disarranged during isotonic contraction, but little change occurs during isometric contraction. The small differences observed indicate that the molecular configuration of the two myosins is not identical but only similar, as is indicated also by the similar but not identical flow-birefringence and solubility properties for myosins extracted from smooth and striated vertebrate muscles (160).

EFFERENT INNERVATION. The majority, if not all, of the organs containing smooth muscle elements receive efferent nerve fibers from both divisions of the autonomic nervous system. However, this is not valid evidence for assuming that all myons are under direct influence of both orthosympathetic and parasympathetic impulses. This can only be proved experimentally, but various investigators have often reported controversial results and conclusions for the same organ of identical species. As the most striking example the dilator iridis may be mentioned, the existence of which is doubted by Langworthy and Ortega (138). The dilator action still present after elimination of any sphincter action they explain plausibly by the diminished blood flow through the iris brought about by arterial constriction caused by the stimulation of the orthosympathetic nerve fibers entering the iris.

For most of the organs, the main difficulty in determining the exact innervation of the muscular elements is due to the well known fact that, as a rule, at least the pre-postganglionic synapses for the parasympathetic division are situated in close proximity to the muscle elements. Furthermore, only for a limited number of peripheral ganglia, in which these synapses are located, has their purely parasympathetic nature been established beyond doubt. Auerbach's plexus of the gut, the largest and probably also biologically the most important peripheral ganglion, is claimed by most textbooks as purely parasympathetic. However, investigators who claim that only parasympathetic synapses are present in this plexus have to admit that orthosympathetic fibers run through the aggregation of nerve cells. In their opinion these fibers do not participate in synapse formation (e.g., 125). Stöhr (223) takes the stand that histologically the nature of the myenteric plexi cannot be claimed with certainty, while Patzelt (176) states definitely that both autonomic divisions participate in the formation of synapses, but that the majority of the entering fibers are parasympathetic.

When nerve fibers, either originating from nerve cells of the ganglia or having passed without interruption throughout the latter, approach the muscle cells, a classification as orthosympathetic or parasympathetic ones is practically impos-

sible, since both are now non-myelinated fibers of extremely small diameter. Tiegs (228, 229) was probably the first who realized that in a number of organs the finest nerve fibers form a true network enveloping the smooth muscle cells and finally entering them. He emphasized the physiological importance of this fact. Such a terminal network has been now well confirmed for the urinary bladder (207, 222), ureter (207), intestinal tract (31, 223, 224, 225), bronchi (119), arrector spinorum of hedgehog (32), iris and ciliaris muscle (33, 35), uterus (208), and blood vessels (32, 34, 223). Stöhr (226) claims that orthosympathetic and parasympathetic nerves unite in the "terminal reticulum" and Boeke (36) also assumes sympathetic and parasympathetic participation in the "terminal syncytium." They use a different terminology for this terminal nerve network, since Boeke regards the interstitial cells of Cajal as an integral part of the network. But since he does not assume any ganglion cell-like activity of the interstitial cells, this difference in opinion is of no interest for the physiologist. After section of the macroscopical nerves, the nerve reticulum shows only minor degenerative changes. The absence of full degeneration might be due to the presence of the interstitial cells. Section of the nerves of only one autonomic division still produces less degeneration. Further confirmations of the existence of such a terminal nerve reticulum were reported by Lawrentjew (144), Reiser (186), Leeuwe (143), and Steffanelli (219), while Clark (66) and Nonidez (170, 171) deny its existence and find the nerves ending on the surface of the muscle cells and not entering them. According to Nonidez the so-called terminal reticulum is in reality a network of argyrophil minute connective tissue fibers, also found in liver, thyroid and other glands. However, this network in glands has been interpreted by Boeke and others as a terminal nerve reticulum. Why these fine connective tissue fibers and not the coarser ones should be argyrophil has not yet been explained by the opponents of the nerve reticulum view; on the other hand it is well known that all neurofibrils are argyrophil. It is understandable that the acceptance of the existence of a true nerve reticulum is difficult for an adherent of the classical anti-continuity neurone theory. That vertebrate axons can fuse to form a nerve net has been demonstrated in tissue cultures (e.g., 14, 15).

This intensive controversy between the histologists has brought out a further fact important for physiological discussion. Contrary to the old belief that nerve endings exist only on a small minority of the individual smooth muscle cells, all recent investigators agree that as a rule nerve endings are found at least for a majority of the muscle cells.

Such a reticulum formation by the efferent fibers explains why in no type of physiological experiment has a true systematization of smooth muscle organs in "motor units" been found. The pilomotors react more or less as a single unit after nerve stimulation and the all-or-none rule is demonstrable to a certain extent (191). This can be explained, despite the nerve net which probably conducts with a decrement as in invertebrates, by the minute spatial dimensions of one single pilomotor and by the probable, but unproved, fact that the nerve net of each pilomotor receives only one postganglionic efferent nerve fiber. It is re-

grettable that no physiologist has used for experimental purposes the much larger arrectores spinorum of the hedgehog. The nictitating membrane, which in certain physiological respects behaves similarly to the pilomotors, is not divided into motor units, and the all-or-none rule does not apply to it (64, 191, 193, 201). Klopp (131) found in experiments with partial denervation of the membrane that localized fractions of the muscles become hypersensitive to epinephrine. This localized influence, for which no sharp demarcation has been demonstrated, does not mean necessarily an organization in units, since in a nerve net conducting with a decrement, efferent fibers connecting with the net will have a more marked influence upon the function of the net around the point of connection than upon the more distant parts. This might explain, too, why stimulation with microelectrodes of the minute nerves leading to small arteries of frogs exerts a spatially limited effect (96). For the dilator iris of the cat, experiments with stimulation of all or only a part of the efferent nerve fibers revealed the absence of true motor units and no evidence even of any spatial preferences (149).

AFFERENT INNERVATION. How far the terminal nerve net is able to carry afferent or antidromic impulses for axon-reflexes is a question which can be answered at present only by conjectures. For all organs containing smooth muscles, there exist true afferent myelinated fibers, which have their nerve cells in the dorsal root ganglia. "Spray like" or "spindle like" sensory endings are found scattered between the myons of the urinary bladder (122, 137), of the sphincter iridis (138) and of the stomach (139) and are probably present in other smooth muscles too. Action currents of the afferent nerve fibers in response to stretch of the muscles have been demonstrated for the urinary bladder of the frog (221) and for the cat bladder and urethra (82).

GANGLION CELL-FREE SMOOTH MUSCLE PREPARATIONS. Unfortunately for the physiological analysis of smooth muscle activity, the synapses in the efferent nerve pathways are not all aggregated in the anatomical ganglia, so that a variable number of true ganglionic synapses are found scattered between the myons. Thus in any analysis of the motor activity of smooth muscle organs, one has not only to ask how far is the observed property a function only of the myons proper or of the terminal nerve reticulum, but one has to consider also the ganglion cells. Absolutely free of nerve cells are the pilomotors (32, 68), the iris muscles (33, 35), the retractor penis (243, 244) and probably the nictitating membrane (195). There is no doubt that in many species, large parts of the uterus do not contain nerve cells (72, 104, 187, 208). Cajal's interstitial cells found abundantly in the uterus and gut are no longer regarded as primitive ganglion cells by most histologists, although a few still maintain the old view (146). The muscularis of arteries is free from nerve cells since the latter are all found in the adventitia (144, 223). In the muscle layers of the gut, a few true ganglion cells are scattered between the myons. But their number is so small that the preparation of ganglion-free strips is possible for physiological experiments, although a later histological examination is indispensable (98, 236, 237). Such strips show nearly as much automatism as strips containing Auerbach's plexus and their reaction to acetylcholine and epinephrine is not altered qualitatively, but only quantitatively. Whether this

small diminution in activity is due to the absence of ganglion cells or due to unavoidable damage during the cumbersome isolation of the strips, is not yet known.

Smooth muscle completely devoid of any nervous tissue is found in the amnion of the chick embryo (83) and in tissue cultures of embryonic gut (204, 227). Both these muscles contract under the influence of acetylcholine (prevented by atropine at least for the amnion muscle) and are inhibited by epinephrine. Euler (80) demonstrated in perfusion experiments on the nerve-free vessels of the human placenta that epinephrine always produces constriction, while acetylcholine influence varies from nil to dilatation or even contraction.

All this evidence indicates that the two chemical mediators can act on the muscle substrate directly, and that neither terminal nerve reticulum nor nerve cells are essential for these reactions. However, the task of analyzing, for these two mediators, their exact sites of action and their relative importance for smooth muscle organs not devoid of nerve cells, is almost impossible. The stimulating action of acetylcholine upon all ganglion cells is well known (56, 64), and that epinephrine can affect the acetylcholine mechanism has been demonstrated recently (62, 220, 230). About the effect of these two drugs upon the terminal nerve reticulum little is known. The well known increase in acetylcholine or epinephrine sensitivity after degeneration of the nerves tells us nothing with certainty about the site of the increased sensibility, since the terminal nerve net undergoes only minor degeneration.

That, even in the absence of nerve cells, the mechanism of the mediator action can be a very complicated one has been shown for the pilomotors. Intracutaneous acetylcholine injections around these adrenergic muscles produce contraction; such a contraction is still produced after section of the orthosympathetic nerves. However, the acetylcholine contraction cannot be elicited after the nerves are degenerated. Coon and Rothman (68) explain these findings by assuming axon reflexes, the receptor end possessing several properties of autonomic ganglia.

ACETYLCHOLINE AND EPINEPHRINE ANTAGONISM. The smooth muscle effectors have often been classified according to their excitatory mediator into adrenergic and cholinergic ones (56, 192). The apparent antagonistic effect of parasympathetic and orthosympathetic nerve stimulation for certain organs has been explained by the presence of two antagonistically arranged muscle sets, one cholinergic, the other adrenergic. For the purposes of this paper a discussion of the differences between sympathin E and sympathin I and of their chemical nature (64, 97, 103, 192) is irrelevant, and, therefore, the expression "epinephrine" is used to designate any adrenergic mediator. Besides the existence of two types of smooth muscles activated by two different mediators, it has been demonstrated that certain smooth muscles are not only excited by one of the mediators but also distinctly inhibited by the other. The best known examples are the gut muscles, for which a reciprocal quantitative antagonism between the excitation by acetylcholine and the inhibition by epinephrine was revealed by Bernheim (28). A similar relation between the effects of the excitatory adrenergic nerve endings and the inhibitory cholinergic ones was found for the retractor penis (173). A large

number of the systemic arteries are contracted adrenergically and relaxed cholinergically (i.e., 156) while on the coronary arteries (i.e., 101) and on the thoracic duct (2) the two mediator actions are just reversed. On the pulmonary arteries, the two mediators act differently but not as true antagonists (79, 91). The sphincter iridis, at least in some species, is under such an antagonistic control of the two mediators (21, 105, 120, 180). For other contractile vertebrate cells, the chromatophores of fishes, the antagonistic influence of mediators or neurohormones is well established (99, 175).

The question of whether those muscles with a well established antagonistic innervation receive nerve fibers from both divisions of the autonomic nervous system (i.e., retractor penis, 173) or only from one division (i.e., 61, 63, 153, 156) is of minor interest from a theoretical point of view, but of practical importance in the analysis of the results of stimulation of a single anatomical nerve.

A further complication in the analysis of the innervation of smooth muscles is the fact that in some organs some of the myons are adrenergic and others cholinergic in the excitation of contraction although no true mechanical antagonism exists between the differently innervated myons. The external layer of the pigeon esophagus is adrenergic and the internal layer cholinergic (111). The stomach muscles of at least some species are partly adrenergic and partly cholinergic (57, 169). In the nictitating membrane, the majority of the muscle cells are adrenergic, but a minority cholinergic (4, 10).

Seasonal, hormonal and vitamin influences. The complexity of the innervation mechanism of smooth muscles, which makes any valid general explanation of excitation and conduction phenomena impossible, is increased by the fact that, even for a given muscle, the mediator sensitivity is fluctuating. The seasonal influence is probably most marked for the smooth muscles of the frog lung (69, 242). The hormonal influence on the activity of the uterus is best known. The effect of the various sex hormones is different from species to species, but can produce in some species an apparent reversal of the effect of nerve stimulation (30, 58, 187). The motor activity of the vesicula seminalis and of the ductus deferens is also affected by sex hormones (157, 158, 235). Urinary bladder (136, 137, 246) and ureter (126, 231, 232) in the females are affected in a comparable manner but less so. Even on the gall bladder (217) and the intestinal tract (84, 206), the influence of the sex hormones is demonstrable. The relation between thyroid activity and sensitivity of the adrenergic mechanism has met with some attention (i.e., 8, 121, 142.) Several of the vitamins can also alter the effective innervation and activity of smooth muscles. Thiamine enhances certain cholinergic mechanisms (1, 53); nicotinic acid influences slightly uterine and intestinal activity (147); and ascorbic acid under certain conditions increases adrenergic as well as cholinergic activity (106, 133).

Action of drugs. A discussion of the various drugs affecting smooth muscles is beyond the scope of this paper, and a number of fairly recent reviews of this subject are available (56, 123, 188, 192). However, a few drug actions must be discussed here on account of their implications for the physiology of smooth muscles in general. Histamine, in more or less physiological doses, contracts

nearly all smooth muscles, the adrenergic and the cholinergic ones. This has been demonstrated for the intestine (25, 26, 27, 28), bronchi (85), nictitating membrane (190), gall bladder (52, 124, 127), uterus (233, 240), iris (108), veins (90, 155), coronary arteries (13), and lung arteries (85, 172). Systemic arteries are contracted too in perfusion experiments with histamine in saline, but the presence of a few red cells and of small amounts of epinephrine changes the contraction effect to dilatation, as shown by Dale and Richards in their classical investigation (71). A similar protective mechanism may be the reason why histamine contraction has not yet been demonstrated for those smooth muscles not mentioned above (i.e. pilomotors). Nearly all investigators agree that the histamine effect is still present after paralysis with atropine or ergotoxin. This can be accepted as evidence that histamine acts neither on the theoretical "receptors for acetylcholine" nor on the "receptors for epinephrine," but on some link closer to the contractile mechanism. It is of interest in this connection that Ambache and Barsoum (7) reported a release of histamine for gut and bladder of guinea pigs and rabbits from muscles contracted by acetylcholine, KCl, or pituitary extracts. This histamine liberation is absent after corresponding treatment of muscles paralyzed by atropine. More observations of this type, if possible also on adrenergic muscles, are needed before far-reaching conclusions can be drawn.

Although not enough data are available to make a general statement, there are indications that angiotonin contracts not only smooth muscles of arteries, but more or less all smooth muscles, and that it acts also on a link closer to the contractile mechanism than the receptors for the mediators. Angiotonin action on the systemic arteries is not prevented, as epinephrine action is, by F 933 or ethyl yohimbine (5, 247). Ergotoxine, however, prevents epinephrine and angiotonin action (241). Strong angiotonin contractions have been demonstrated on the gut (67, 112, 174), uterus, bladder and nictitating membrane (5, 152). Distinct, but not quite such strong angiotonin effects were observed for ureter, gall bladder, ductus deferens and bronchi muscles by Ludueña (152).

The well known paralyzing effect of atropine upon cholinergic myons has one feature which must be discussed here. For some muscles, atropine prevents only the effect of externally or intravenously applied acetylcholine but diminishes very little or not at all the effect of parasympathetic nerve stimulation (70, 122, 123, 169). The current explanation that atropine prevents diffusion of acetylcholine through cell membranes but does not prevent intracellularly liberated acetylcholine from reaching the receptors, assumes that in those muscles in which atropine blocks nerve impulses, nerve endings exist only on the surface of the muscle cells, while in those muscles in which no nerve block occurs, all nerve endings are intracellular. For those muscles with partial nerve block produced by atropine, it is believed that some of the nerve endings are intracellular and some extracellular. Corresponding observations and conclusions have been made for the effect of ergotoxine upon excitatory adrenergic muscles, where also apparently diffusion of epinephrine into the cells is prevented, but not mediation from an intracellular nerve ending (i.e., 64, 129, 190, 192). The explanation that only a

limited number of muscle cells is innervated at all and that normally the impulse is transmitted from the directly innervated cells by diffusion to the non-innervated cells can hardly be maintained in view of the present histological evidence mentioned before. Unfortunately, the question of histological and physiological innervation of a smooth muscle organ has never been investigated by a combined team of histologist and physiologist, both well acquainted with the methodological difficulties of the problem.

Some doubt about the value of atropine and ergotoxine as tools for exploring the excitatory cholinergic or adrenergic nature of smooth muscle innervation has been expressed by Mellanby and Pratt (161, 162), who believe that at least some of the paralyzing effect is not due to a specific blocking but to a general "immobility" of the muscle substance proper caused by these drugs. Also some experience makes it doubtful that the only effect of ergotoxine is a blocking of excitatory adrenergic mediation (i.e., 4, 169, 151, 205).

Sensitization of denervated smooth muscle to its mediator. After section of the postganglionic nerves, smooth muscles do not degenerate but show always an enormously increased sensitivity for their excitatory and their inhibitory mediators (70, 110, 150, 210). After regeneration of the nerves, the mediator sensitivity declines again to normal values. Section of the preganglionic nerves produces much less sensitization (212) or no sensitization at all (248) according to which muscle is used in the experiments. Increased permeability of the muscle cell membranes is generally accepted as the mechanism of this sensitization for the specific mediators (64). This also explains why simultaneously the sensitivity is increased for drugs which act on links, which are closer to the contractile mechanism than are the receptors for the mediators (e.g., histamine, 190).

Electrical excitability. One of the outstanding features of the postganglionic-denervated nictitating membrane is the apparent absence of electrical excitability (34, 75, 167); this excitability can be restored, to a certain extent, by treating with the drug F 933. Unfortunately only few smooth muscles allow complete postganglionic denervation. The rectractor penis, probably very suitable for this type of experiment, has apparently not been used. Pilomotors are completely unexcitable electrically after denervation (196). Chronic denervation is at the moment the only experimental condition in which the electrical stimulus might act on the muscle cells alone, if the slight degeneration of the nerve reticulum excludes stimulation of it. The intimate relation between terminal nerve reticulum and muscle cells excludes the use of micro-electrodes, and the blocking by atropine or ergotoxine does not affect intracellular nerve endings. The nerve-free muscle cells of the chick amnion are electrically excitable, but rather strong currents are apparently needed (179).

Chronaxie. Nearly all chronaxie measurements have been made on organs or on strips of organs containing smooth muscles besides various nervous tissues. Therefore it is more or less guess work if one attributes the measured chronaxie either to the muscle cells proper or to the nervous elements. The chronaxie values reported are all rather long, at least 15 times as long as for skeletal muscles of the same species (37, 43, 81, 188), and much longer than the chronaxie values

for the slowest vertebrate nerve fiber yet measured. However, the diameter of the nerve fibers forming the terminal reticulum is also much smaller than that of the smallest nerve fiber subjected up to now to direct chronaxie measurement. The strength-capacity curves determined for the nictitating membrane (213) revealed typical breaks into an α and a γ segment. Again, it is more or less arbitrary whether one ascribes the higher α excitability to nerves and the γ excitability to the muscles proper, or whether one assumes that the higher excitability is that of the somewhat larger nerves leading to the nerve net, and the lower excitability is that of the finest fibers forming the terminal reticulum. No break in strength-duration curves was observed for the ureter of rabbits and for the uterus of rabbits, guinea pigs, and cats by Bozler (43). He regards the strips of these organs as true muscle syncytia like the heart, since they follow the all-or-none rule and since conduction is not much disturbed by zigzag cuts. However, all these observed properties can be ascribed as well to the terminal nerve reticulum (228, 229).

For some smooth muscles, the chronaxie varies with the season (94). In some species the uterus is not electrically excitable in anestrus but excitable in estrus, and it can be demonstrated that chronaxie shortens with increased influence of estrogens (43, 130). It would be of value if, on the same muscles, the changes brought about by seasonal and hormonal influences in chronaxie and in mediator sensitivity could be studied simultaneously.

Electrical stimulation with variation in frequency and slope of the current shocks has not yet been reported as stimulating selectively either the excitatory or the inhibitory mechanism of isolated vertebrate smooth muscles responding antagonistically to the two mediators. However, on single nerves leading to such structures selective indirect stimulation of the excitatory and the inhibitory mechanism has been possible (156, 209, 238). According to Lapicque (140), all autonomic nerves are "iterative," which means that only a summation of arriving volleys can elicit the reaction of the effectors. At least for the excitatory nerves of the nictitating membrane, of bladder and of pilomotors, it could be demonstrated that a single nerve volley can produce contraction (55, 76, 199). The same has been found for the excitatory vagal fibers leading to esophagus and stomach of the cat (154). For no inhibitory nerve of vertebrate smooth muscle has such a single-volley effective yet been demonstrated, but the inhibitory vagal heart mechanism is not "iterative" (86, 100).

CONTRACTION TIME AND LATENT PERIOD. One of the few general physiological characteristics of vertebrate smooth muscles is the slowness of contraction. Smooth muscle contractions elicited by single indirect or direct stimulation last at least 15 times as long as a twitch of any striated muscle of the same species (20, 135, 154, 213). The ratio between contraction time proper and relaxation time is always lower than in striated muscles. Thus relaxation is as a rule a very slow process, a fact which must be kept in mind for any discussion of the tonus problem. For those smooth muscles with inhibitory innervation or inhibitory mediator mechanism, relaxation can be speeded up and might become quicker than the contraction phase (41, 42).

Due to the intimate anatomical relation between nervous tissue and muscle cells and due to the slowness of the mechanical process, any determination of the mechanical latent period for smooth muscle organs has limited value only. This latent period, which lasts at least 0.15 sec. but often reaches much longer values (135, 154, 199), varies not only from organ to organ and species to species, but also for different parts of the same organ (i.e., intestine, 6). If the postganglionic nerves of the nictitating membrane are stimulated by single shocks, the latent period declines from 0.4 to 0.15 sec. with increasing strength of the stimuli (76).

Refractory period. In experiments with mechanical recording, the refractory period can be determined only as the minimal interval between two successive stimulations still producing summation. For the stomach of the cat, McSwiney and Robinson (154) report a refractory period of 0.8 sec., while for the nictitating membrane, Brown (55) reports a refractory period of 2 msec., which was more or less confirmed by Rosenblueth and Acheson (194), while according to Eccles and Magladery (76), as judged by electrical evidence, some delay in summation occurs for intervals shorter than 55 msec. This large difference between refractory period of the stomach and that of the nictitating membrane might find an explanation in the observation of Umrath (234) that two different refractory periods can be demonstrated on the frog rectum. The "autogenous" refractory period, that is, that of the muscle cells themselves, is very short, less than 10 msec., and is lengthened after two excitations and by fatigue or narcosis. The "induced" refractory period, which is forced upon the colon by Auerbach's plexus, acting as "pacemaker", lasts about 20 sec. and is shortened after two excitations and by fatigue or narcosis. It must be pointed out, however, that ganglion-free muscle organs also can have rather long refractory periods (e.g., ureter, uterus, 43). Has the induced refractory period been measured in those muscles, and is the terminal nerve reticulum (or the interstitial cells) responsible for the induced refractory period? Since in such muscles pacemaker functions have been demonstrated (50), a positive answer to these two questions might be permissible.

Conduction. Monnier (166) demonstrated for isolated arteries that contractility and conduction are, at least for these muscle organs, not identical processes since conduction can occur without contraction of parts of the artery through which conduction has taken place. In the retractor penis, local electrical stimulation produces only more or less localized contraction (243). On the same muscle, mechanical stimulation applied strictly locally without stretch of other muscle parts, also produces only local contraction (88). On the other hand, there is no doubt that some smooth muscle organs not containing ganglia show conduction. For oviduct, ureter and uterus (in estrus) conduction rates between 2 to 60 mm/sec have been found (43, 49, 116, 168). It is possible, too, that in some organs under certain conditions, e.g., uterus, excitation by nerve stimulation is localized and non-conducted as is chemical stimulation, while direct electrical stimulation elicits a conducted process corresponding to that occurring during spontaneous movements (193).

ACTION POTENTIALS. Not only because of the diversity in anatomical arrangement and in function of the various smooth muscle organs, but also because of certain methodical difficulties in recording electrical potentials and their changes, the findings and the interpretations of various investigators are rather controversial. The majority of the investigations published before 1935 were made without amplification, with string galvanometers; the strings were very loose to secure the needed high sensitivity. With such a method, potential changes of slow frequencies can be recorded more or less correctly, but not those of relatively high frequencies. The use of amplifiers in connection with various recording instruments permits the recording of high frequency changes of minute potentials, but does not allow the accurate recording of slow potential changes. Furthermore, many investigators using amplifier technic, did not record simultaneously the potential changes and mechanogram. Thus, any time relation claimed between potential changes and contraction becomes more or less arbitrary.

As mentioned above, one of the relatively simply organized smooth muscle organs is the *pilomotors*. However, even their potential changes, occurring after single shock stimulation of their nerves, are rather complicated and vary a good deal (135, 198, 200, 201, 202). Of the seven different potential waves observed, only the four main ones will be mentioned here. The first two are rapid potential changes in quick succession. Potential II slightly precedes or is simultaneous with the onset of contraction. Potential IV is a slow wave which follows closely the course of contraction, but is not an artefact due to contact changes on the electrodes or due to changes in shape of the tissue between the electrodes. Potentials III are some medium quick waves, not always present, superimposed upon the slow basic potential IV. The presence of such superimposed waves upon the contraction potential is not surprising since the mechanogram of the pilomotors often shows some superimposed oscillations (20). Potentials I and II increase upon repetitive stimulation, while I, III and IV, but not II, disappear after ergotoxine paralysis. Epinephrine in small doses, which do not contract the non-sensitized pilomotors, decreases potentials I and II. If the pilomotors are sensitized by cocaine, the same dose of epinephrine produces contraction, and the depressing action of epinephrine upon potentials I and II elicited by nerve stimulation is enhanced.

The *nictitating membrane* gives rather similar records (135, 198, 200, 201, 202). The main difference is that the potential change occurring parallel with the contraction can be subdivided much more clearly and constantly into the basic potential IV and the superimposed quicker potential III, which is of varying character: monophasic, biphasic, polyphasic, or distinctly repetitive during the whole contraction. With repetitive stimulation of the nerves, potential I usually shows a decline, while II becomes more marked. Cannon and Rosenblueth (192), assuming nerve endings on only a limited number of muscle cells, tentatively identified the potential I with depolarization of the directly innervated key muscle cells, potential II with depolarization of adjacent non-innervated muscle cells, and potential IV with depolarization of other interfaces and with contraction. Eccles and Magladery (76, 77) more or less confirmed all these findings, but ob-

served a further small wave N preceding potential I. N is even present when the nerve stimulus is too weak to produce contraction and potentials I and II. The interpretations of Eccles and Magladery, however, are quite different, since they believe in the existence of a fairly long refractory period of the muscle cells and in the conduction of a wave of negativity, propagated distally along the membrane at a rate of 50 to 80 mm./sec., which they regard as the expression of muscular conduction. Rosenblueth (193) emphasizes rightly that the electrical potentials of smooth muscles are too complicated and not sufficiently understood to draw conclusions concerning the properties of smooth muscles contradictory to other evidence. The anatomical arrangement of the muscle cells in the nictitating membrane renders muscular conduction rather unlikely (3). Reinvestigation of the potentials with special search for any indication of conducted negativity waves or of refractoriness of the muscles confirmed their absence (202). During epinephrine contractions, the potentials observed resemble very much potential III and IV after nerve stimulation (77, 167, 198, 199). Nerve stimulation, while the membrane is under the influence of epinephrine, produces electrograms in which the potentials I and II are diminished or even completely absent (64), although the muscle still responds with further contraction.

For the *urinary bladder* of the cat after stimulation of the pelvic nerves, potentials rather similar to those for pilomotors and nictitating membrane were recorded (135, 199), and are similarly interpreted as of local origin and not conducted.

It is regrettable that the potential changes of the *retractor penis* apparently have never been studied with modern technic, since this muscle is very suitable for studying the problem of conduction. The string galvanometer record of Brücke and Oinuma (59) shows indications of small spikes and large slow wave components, the latter probably comparable to potential III.

The ganglion cell-free *ureter* of the pig shows regular contraction waves starting from the renal end, which are accompanied in string galvanometer records by mono- or di-phasic potentials according to the placement of the electrodes (115). Each wave lasts for several seconds, and their frequency varies between 2 and 8/min. The amplitude and frequency increases from renal to vesical end. Amplitude of the potential and strength of contraction show no strict correlation. Bozler (44, 49, 50, 51) reinvestigated the ureter of various species with the amplifier technic and found, with a monophasic lead for the guinea pig, discharges up to 40/sec. during a peristaltic wave. However, in the rabbit and especially in the rat, the action potentials are rather slow negativity changes (lasting up to 3.5 sec. in the rat). Bozler is inclined to explain these long-lasting action potentials by a burst of impulses fused into a continuous state. At the pacemaker of the renal ureter end, Bozler observed small local negative potentials developing before the outburst of conducted impulses. Isometric, but not simultaneous, recording of the mechanogram revealed a small tonus increase preceding the start of a contraction at the site of the pacemaker. According to Bozler, both the tonus increase and the small negativity are expressions of a local depolarization.

During the spontaneous contractions of the *ductus deferens* of cats, waves of negativity, more or less parallel in time with contraction can be detected with the string galvanometer (9). In corresponding experiments slow mono- or di-phasic potentials, lasting 3 to 9 sec. each, have been found in rabbits (117).

The contractions of the *vagina* of rabbits give negative potential waves of 5 to 12 sec. duration coinciding with contraction (38, 39). Similar waves lasting about 9 sec. and travelling at a rate of 1.7 to 3.3 mm./sec. have been observed on the isolated *oviduct* of pigs by Hasama (116). He believes that the occasionally recorded superimposed waves of higher frequency (2/sec.) are due to "interference" of the potentials of parallel muscle fibers.

The electrical activity of the *uterus* is complicated by the already discussed influence of the sex hormones upon excitability and conduction. With the string galvanometer, slow negative potential waves were observed by Hasama (113, 114) on the virgin rabbit uterus with a frequency of 3 to 7/min., more or less synchronized with contraction waves. However, there was no constant parallelism between amplitude or frequency and the strength of contraction. In the isolated uterus, oxygen lack depressed the potentials much less than the contractions. As a rule, sympathetic drugs increased amplitude and frequency of the potentials, while parasympathetic drugs only increased the frequency. For the cat, essentially similar potentials were recorded by Bacq and Monnier (9). Epinephrine increased the potential waves and the contractions of the gravid uterus, but inhibited both phenomena in the nongravid uterus. In the latter, potentials and contractions were increased by acetylcholine.

Although Greene reported in 1928 in a short note (102) that, besides the slow waves of the uterus, there also occur rapid repetitive deflections, our knowledge of the electric activity of the uterus was really clarified only 10 years later by the investigations of Bozler. The latter not only demonstrated for the guinea pig, rabbit and cat that conduction and electric discharges are dependent on a certain minimal level of estrogens (43, 48), but he was also able to record rather quick potential waves by the use of amplifiers (44, 46, 47, 51). Unfortunately, the contractions were not recorded simultaneously. These high frequency waves can be obtained from the conducting uterus either monophasicly or diphasically. The conduction rate is slow for the guinea pig (1 to 3 mm./sec.), but higher in the two other species (up to 60 mm./sec.). The single waves have varying shape but are all rather short, and bursts of impulses up to 40/sec. were observed. Sympathetic nerve stimulation or epinephrine lowers or abolishes the potential waves and their conductivity in the non-pregnant uterus of the cat, but this inhibitory effect is often preceded by a short excitatory state. Bozler assumes that this diphasic response is due to a diminution in excitability which leads to a block of muscular conduction, but that simultaneously new local impulses are set up. The excitatory action will be suppressed as soon as the excitability level has dropped sufficiently to block conduction. Morison (168) confirms these results in the main and emphasizes the local action of epinephrine and of nerve stimulation. During prolonged epinephrine contractions, not only the slow waves are present but also the quick, spike-like potentials. The slowing action of epine-

phrine in the non-pregnant cat uterus is only present if the drug is applied to the pacemaker at the uterus horn. Balassa (11), using the string galvanometer and recording simultaneously the contractions, observed for rabbits in anestrus that slow potential waves occur with the weak contractions, and that the more spike-like potentials are only present during the more active contractions during estrus or late pregnancy. For the cat, Balassa and Gurd (12) also report slow waves in anestrus, but for estrus and late pregnancy, high frequency potentials in short bursts occurring only at the start of spontaneous and of drug contractions. They assume with Bozler that only when the estrogenic hormones predominate is conduction from muscle cell to muscle cell possible. For the human, and also for the dog, high frequency potentials during spontaneous and pituitrin contraction of the uterus have been observed (128).

Puestow (181, 182), as well as Berkson (22, 23, 24), reported electromyographic studies of the *intestine* in situ or after isolation of the rabbit, dog, cat, guinea pig and rat. With the string galvomometer without amplifier, monophasic or biphasic records are obtainable; they are rather variable even for the same species. However, in all cases a slow negative wave occurs parallel with the mechanogram during a peristaltic wave. Rapid spike-like potentials of various shape are superimposed on the basic potential wave during the contraction proper. Such rapid potentials are present, too, during long-lasting contractions produced by direct repetitive electrical stimulation. However, occasionally complete spontaneous dissociation of the electrical and mechanical activity occurs in apparently normal loops. Curare increases the strength of spontaneous contractions without altering the electrogram. Epinephrine paralyses the loops without abolishing the electrical activity completely. Eserine increases the motor activity and the number and frequency of the spike potentials. Nicotine applied to an isolated loop produces at first strong contractions with quick electrical oscillations, but after return to normal motor activity, not only the quick but also the slow electrical waves are absent. Nicotine applied to a loop in situ has little effect upon the spontaneous contractions, but abolishes all the electrical signs if the circulation of the loop has been diminished by arterial compression. Restoration of circulation will restore normal electrical activity with little change in the mechanogram. Puestow and Berkson conclude that the electrical potential changes originate within the intrinsic nervous plexus. The slow potential waves were reported, too, for the rabbit stomach (118). Paulian and Felice (177) claim that in the cat intestine the onset of the slow negativity wave precedes spontaneous and also acetylcholine contractions, but no tracings are given in their paper.

Bozler (44, 45, 46, 49, 50, 51) recorded with amplifier, but without simultaneous mechanogram, the potential from isolated intestinal strips and from loops in situ for the dog, rabbit, cat, guinea pig and rat. No slow potential waves were observed in his experiments, but occasionally long-lasting after-potentials. Although the electrograms varied not only from species to species, but also from one type of contraction to another (pendulum movement, peristalsis, etc.) there was one common characteristic; namely, the presence of bursts of repetitive dis-

charges of spike-like potentials. The frequency ranged from 1/sec. for the cat stomach to 10/sec. for a peristaltic rush of the small intestine of the rabbit. These quick potentials can be recorded either as monophasic or as biphasic ones and are conducted waves of negativity. Bozler believes the potentials are too large to be produced by the relatively small amount of nervous elements present, and assumes muscular origin and muscular conduction through protoplasmic bridges from muscle cell to muscle cell. Epinephrine, in doses not high enough to stop the intestinal movements completely, does not diminish the frequency of spontaneous movements, but only their strength, and the number of electrical discharges found is accordingly diminished. Bozler explains this by a lowering of the average level of excitability by epinephrine. As for the pacemaker of the ureter, slow local potential changes are almost continuously present in all regions of the intestine. Impulses, often in bursts, are discharged at the crest of such local negativity waves. Forster and co-workers (92) were able to demonstrate bursts of initial spikes at the onset of contractions in the human intestine; these are followed by a long-lasting base line shift with superimposed intermediate electrical activity.

For the *gall bladder* of rabbit and guinea pig, slow potential changes and quicker superimposed spike potentials have been recorded with the string galvanometer (16, 17, 18, 148). The slow waves occur more or less simultaneously with tonus changes, while the bursts of spike potentials occur during true contractions.

The diversity of the electrograms from various smooth muscle organs makes it impossible to propose any unitary theory for the origin and mechanism of these different action current components. There is no doubt that for some of the organs, the spike potentials behave rather similarly to those in skeletal muscles, and that for these smooth muscles, tonus could be explained by gradation in number and in frequency of the impulses (48). It does not matter much for this question if the sites of origin of these conducted potentials are nervous elements or the muscle cells themselves. But the fact that complete dissociation between electrogram and mechanogram can occur indicates that the mechanism, which finds its expression in the spike potentials, is probably only of regulatory importance. The existence of localized potentials occurring at least in some smooth muscle organs is evident. The potentials III and IV of pilomotors and the nictitating membrane are not identical with the slow waves in the intestine, uterus and ureter since the latter are conducted. However, there is the theoretical possibility that these slow potentials are localized potentials of the individual muscle cells, thrown successively into activity by conduction through the terminal nerve reticulum and thus simulating a conducted wave of negativity. It might not be advisable, since the evidence is not strong enough at present, to generalize with Monnier (165) that depolarization of the muscle cells themselves is constantly associated with contraction, and increased polarization with relaxation.

Impedance changes. Paulian and Felice (177) tried to support the depolarization theory by measurements of the impedance of the isolated cat intestine. They claim that impedance decreases with contraction and diminishes with

relaxation. However, as pointed out elsewhere (87), the resistance to alternating currents of high frequency of a muscle fiber is not determined, at least theoretically, by the characteristics of the membrane alone but also by the submiscroscopical structure of the muscle fibrils changing with contraction and relaxation. Careful impedance measurements on simple smooth muscle organs would be valuable for increasing our knowledge about the mechanism of smooth muscle activity.

ELASTIC AND MECHANICAL PROPERTIES. The differences in the length-tension curves for striated and smooth muscles, for the resting state as well as for the contracted state, have been regarded for a long time as one of the main distinctions between the physiological characteristics of these two muscle types. But for the last 20 years it has become more and more clearly recognized that all contractile tissues give qualitatively similar curves, the main difference being only in the time factor (189). If resting vertebrate smooth muscle organs are stretched slowly, the tension increases only a little but linearly for a considerable range, and only for extreme length can considerable tension be produced by slow elongation (retractor penis, 243; blood vessels, 65; uterus, 47, 211; urinary bladder, 60). That the isolated striated vertebrate muscle fiber has also over a long range a constant extension modulus is now well recognized (184). According to Winton (245), the relation between tension, length and time for a stretched or released retractor penis can be represented by a mechanical model including a pure elastic resistance, a viscous-elastic system, and a pure viscous system arranged in series. There is no doubt that the absolute values to be assigned to these three components differ a great deal from one smooth muscle organ to another (214, 215). Bozler (48) demonstrated for the muscle coats of the arteries and for the uterus that the rate of extention under a load follows the same time course as the lengthening after a contraction. He concludes that relaxation, if not speeded up by an inhibitory mediator, is a purely physical process controlled alone by the visco-elastic properties of the contractile elements.

For a number of smooth muscles (intestine, 54; retractor penis, 243; nictitating membrane, 109; urinary bladder, 60), it has been demonstrated that the maximal tension increment produced by electrical stimulation or mediator action increases with the resting length of the muscle up to an optimal length, beyond which a decrease in tension increment occurs. This is exactly the same tension-length relation as for skeletal muscle (183). As in the latter, at least for some smooth muscles, this optimal length is beyond the maximal physiological length. The tension-increase with length apparently does not apply for autogenous rhythmic contractions (urinary bladder, 161, 162; uterus, 211), probably due to a depressing effect of stretch upon the pacemaker. Only one investigator has tried to determine the "absolute muscle force" (maximal tension increment per cm.^{2} cross section at optimal muscle length). Ducret (73) gives a value of 800 gm./cm.^{2} for the epinephrine contraction of mesenteric arteries. Using tension and dimension values given by the authors or assuming the probable size of the muscle strips, the absolute muscle forces can be calculated as 650 gm./cm.^{2} for the indirectly stimulated nictitating membrane (109), 630 gm./cm.^{2} for the histamine

contraction of the cat ileum (54), 440 gm./cm.^{2} for the electrically stimulated and 650 gm./cm.^{2} for the epinephrine contracted retractor penis (243). These values are much lower than those for most striated muscles (89), but their conformity is astonishing. However, more data are needed to allow any general conclusion.

As in striated muscle, the tension increment of smooth muscle is affected not only by the muscle length but also by the experimental temperature (185, 244). Hydrostatic pressure affects the tension response of smooth muscles rather similarly to that of striated muscles, and very high pressure produces a compression contraction with a tension surpassing that for maximal electrical stimulation (74, 78).

"Tonus." The change in our general attitude towards the tonus problem as far as it concerns smooth vertebrate muscles is best illustrated by a comparison of two papers of Bozler, one published in 1928 (40), the other in 1936 (42). In the first paper he tries to demonstrate that also in vertebrate smooth muscles, the coarse and thin fibrils existing side by side in the same cell have different functions, tonic activity and quick contraction respectively. In the second and in later papers, he states emphatically that there does not exist any specific tonic mechanism and that tonus can be explained perfectly by the normally slow relaxation of smooth muscles, which can be speeded up at least for some smooth muscles by the action of an inhibitory mediator, a mechanism not existing in striated muscles. Bayliss (19) pointed out as early as in 1928 that, in consideration of the slow relaxation of smooth muscles, tonus can be maintained by continuous excitations with little expenditure of energy. The measurement of the heat production of vertebrate smooth muscles during so-called tonic contractions is a task which nobody familiar with the pitfalls of our present methods has yet felt inclined to attempt. For the relatively quick contractions of the cardiac sphincter of the turtle stomach following vagal stimulations, Snyder (218) found after applying various correction factors that the ratio of the heat to the product of tension, weight, and time is of the same order of magnitude as in striated muscles. Although there is no doubt that all our recent experimental results favor a unitary conception for the quick and the tonic contractions, the last word concerning the tonus problem of smooth vertebrate muscles has not yet been written.

Conclusions. Vertebrate smooth muscle organs show a large diversity of physiological characteristics. However, all phenomena closely associated with the contractile mechanism proper are common to all smooth muscles and are rather similar to those of striated muscle; the time factor alone is much shorter in the latter. While for striated muscles only one unitary mediator mechanism eliciting the contractile discharge exists, smooth muscles can be classified into two groups according to the excitatory mediator involved. Furthermore, for a number of smooth muscles there exists also an inhibitory mediator. For these muscles, the strength and the time curve of activity are determined by the spatial and temporal interaction of the two antagonistic mediators. For some smooth muscles, activity might be controlled by the combined action of more than two

physiological mediators. Due to the smallness of a smooth muscle cell, the integration of the activity of the single cells into the function of the whole smooth muscle organ requires a more complicated mechanism than that for integration of single striated fibers into the activity of an anatomical muscle. Diffusion of the mediator is apparently only of minor importance under physiological conditions. In some smooth muscle organs there exists a mechanism for conduction. Whether the latter occurs through protoplasmic bridges of the muscle substance proper or through the terminal nerve reticulum, cannot be decided yet, and difference in this respect might be present from one organ to the other. There is no doubt that some smooth muscle cells are capable of autogenous impulse or pacemaker activity. But this does not mean necessarily that all autonomic activity of smooth muscle organs is of myogenic origin. The autonomic impulse mechanism of the cardiac ventricle comes into play, as a rule, only when the conduction system is interrupted. Even striated muscle fibers show automatic impulse formation after denervation, but nobody would conclude that the impulses for normal skeletal muscle activity are myogenic. In the majority of the smooth muscle organs, activity is controlled and governed by extrinsic nerves and by the intrinsic ganglion plexi, thus probably suppressing any automatic impulse tendency of the muscle cells proper, if existent. The correlation between autonomic nervous system, mediator action, and contractile mechanism is in some smooth muscle organs dependent on the balance between the levels of various hormones.

Since the contractile mechanism in smooth muscle is so similar to that of striated muscle, where it can be studied under much more favorable conditions, the main task for the future concerning the physiology of vertebrate smooth muscles will be the analysis of the mechanisms by which contraction of the single muscle cells are integrated into the specific functions of the various smooth muscle organs.

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WATER EXCHANGE

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Some years ago the author (317) attempted a comprehensive treatment of the functions of water in biological reactions. This article must lean heavily on that earlier volume and on previous reviews, confining itself largely to recent contributions.

MEMBRANE EQUILIBRIA AND TRANSFERS OF WATER WITHIN THE BODY. Despite the extreme differences in composition of the contents of its various compartments, a uniform osmotic pressure prevails throughout the fluids of the body. It follows that the membranes between these compartments must universally permit the free passage of water and that exchanges of water among the compartments must be determined by the hydrostatic and osmotic forces within them.

Movements of water between the circulating blood and the extracellular fluid. The demonstration, by direct analysis of edema fluid and other transudates and by inferential methods that the extracellular fluid has the composition of an ultrafiltrate of plasma, is more substantial proof of the validity of the Starling theory than are quantitative data which show that the colloid osmotic pressure of the plasma and the capillary blood pressure have the proper relative magnitudes (259, 382). The effective hydrostatic force tending to expel fluid from the capillaries is the capillary blood pressure minus the tissue pressure (i.e., the elastic resistance of the extravascular spaces to distention); the effective osmotic force which tends to drive water back into the blood stream, the colloid pressure, is the osmotic pressure of the serum proteins minus the osmotic pressure of the proteins in the perivascular fluids.

The colloid osmotic pressure of the proteins of normal plasma, including a contribution from the Gibbs-Donnan effect, amounts to about 30 cm. of water or 22 mm. of mercury (436). The greater part of the colloid osmotic pressure derives from serum albumin, which has a smaller molecule than globulin. The capillary blood pressure varies greatly according to its anatomical situation and local circulatory reactions. In those parts of the general circulatory system that have been investigated, the pressure at the arterial end of the capillaries appears to be greater, that at the venous end less, than the colloid osmotic pressure (259).

The impermeability of the capillary walls to protein is not continuous, absolute or uniform. McCarrell, Thayer and Drinker (297) have verified Starling's (382) observation that the concentration of protein in lymph from the liver is practically identical with that of plasma. If the interstitial fluid about the portal capillaries did not contain high concentrations of protein, exchanges of fluid in this region would be impossible because in the portal vein the blood pressure is lower than the colloid osmotic pressure of the plasma proteins. Although the proteins in hepatic lymph may be partly formed *in situ*, the rapid

appearance in thoracic duct lymph of foreign protein (281) and of dyes that adhere to proteins (62, 127, 364), after intravenous injection of these materials, indicates that proteins must escape readily from the capillaries of some regions drained by the thoracic duct. The low concentrations of protein in edema fluids and transudates, 0.06 to 0.5 per cent, (179, 259, 317, 347, 410) and the failure of dyes to enter these fluids in appreciable quantities (127, 160) denotes that the capillaries of peripheral tissues are far less permeable. Foreign proteins and dyes also appear in smaller quantities in lymph draining the extremities than they do in thoracic duct lymph. In the latter, for example, after injections of the blue dye, T-1824, Courtice (78) and Ferrebee et al. (127) found dye concentrations as high as 30 to 40 per cent of those in plasma with a lymph flow of 100 to 200 cc. per hour while in cervical lymph the concentrations were only 3 to 15 per cent of those in plasma with a lymph flow of only 10 to 20 cc. per hour. Anoxia (258, 291) and vasomotor disturbances induced by physical agents (253, 260) or drugs (39, 172, 384, 394) can increase capillary permeability so much that proteins escape freely from the circulation. Fluid of urticarial wheals or angioneurotic edema contains high concentrations of protein (173, 347). Such lesions also take on an intense blue after injections of T-1824 (127).

Tissue pressure also varies with structure and location. In the subcutaneous tissue of a normal subject in the recumbent position, at the level of the heart, it is usually less than 5 cm. of water, occasionally as high as 7 to 8 cm. (53, 293, 376, 431). It rises when venous congestion is induced, because of the expansion of the veins (431), and is also increased by accumulation of edema fluid (376). At rest in the recumbent position, Wells and Youmans (431) found intramuscular pressures from 2 to 11 cm. of water, highest in muscles with tight fascial sheaths. When venous pressure was raised to 100 cm. the intramuscular pressure in the tibialis anticus rose as high as 50 cm. of water. The relatively small expansibility of muscles may explain the comparative immunity from edema that they seem to enjoy.

The volume of the circulating blood. The retention in the blood stream of dyes used to measure plasma volume depends not upon their molecular size or other inherent physical properties, but upon their propensity to combine with serum protein, especially the albumin fraction (331). Like the latter they are not entirely restrained by the capillary walls. Large amounts of T-1824 or brilliant vital red appear in thoracic duct lymph shortly after either is injected into the blood stream (62, 127, 364). If they were steadily, gradually and permanently removed from the circulation by this route from the moment of their injection, the true volume of the plasma could be estimated by extrapolating to the moment of injection the curve describing the decrease of dye concentration in the blood plasma. There is no certainty, however, that the dissemination of dye through the circulating blood and its escape into thoracic duct lymph are either initiated or reach steady states simultaneously. The return of dye to the blood stream again via the thoracic duct defies correction. The amount of dye returned may ordinarily be so small, as Courtice claims (78), that the error of including a shunt in the system is of no great significance. Variations in the volume and flow of thoracic duct lymph, which may occur under the very conditions in which

knowledge of plasma volume changes is most desirable, must introduce incommensurable errors of no negligible magnitude if, as Ferrebee, Leigh and Berliner (127) have estimated, the thoracic duct lymph system may contain as much as 7.5 per cent of the dye injected. As evidence that removal of dye from the blood stream is not a uniform process, Gilder, Müller and Philips (163) found that extrapolation of the disappearance curve to zero time shortly after mixing is complete and extrapolation at a later time yield different values for plasma volume.

According to most observers the carbon monoxide method, which measures blood volume directly, yields smaller values than the dye method does (29, 365). In normal subjects Hopper (226) found that the two methods agreed on the average, but differed in one direction or the other in individual subjects. It seems highly unlikely that some of the carbon monoxide is absorbed by myohemoglobin or that it penetrates to cells in repositories such as the spleen (226). It seems more likely that technical errors, all of which will tend to give excessive values, cannot be excluded and that, as Whipple and others (183, 365) have suggested, the red blood cells are not evenly distributed throughout the circulation. Transfusions with red cells containing radioactive iron or phosphorus (62, 181, 182, 183) are not practical for most purposes.

Both carbon monoxide and dyes appear to give too large values for blood volume in normal persons, which may be greatly and unpredictably magnified by physiological and pathological disorders. The carbon monoxide method, though technically more difficult, is probably less subject to gross errors because red blood cells are less prone than proteins and dyes to escape from the circulation (145, 226). Hopper (226) found that while the two usually agreed well in normal subjects and in many disordered states, the dye method occasionally indicated paradoxical expansions of the plasma when the carbon monoxide method and all other criteria indicated hemoconcentration.

The most extensive series of measurements, by Gibson and Evans (161), by a refined technique with the blue dye T-1824, gave blood volumes for normal resting adults of 2990 to 6980 cc., averaging 4635 cc. Related to weight the average was 72.4 cc. per kgm. with extremes of 46.3 to 97.7 cc. Related to surface area, with which the correlation was somewhat closer, the average was 2750 cc. per sq. m. There was a distinct sex difference, the average for males being 77.7 cc. per kgm. (range 66.2 to 99.7), for females 66.1 cc. per kgm. (range 46.3 to 85.4). This difference is not, as Chang and Harrop (61) earlier suggested, referable to differences in body build (161, 226). In the series of Gibson and Evans (161) the average for males was 2930, for females 2530 cc. per sq. m. The greater volume of blood in males depends chiefly upon the larger proportion of cells. In adults the ratio of blood volume to size varies little with age. Immediately after birth infants may lose considerable fluid with concomitant hemoconcentration (19) which rapidly disappears when adequate fluids are taken. In the first weeks of life the cell volume falls while the plasma volume remains constant (20). Both blood and cell volumes are greater in proportion to body weight, but smaller in proportion to surface area, in children than in adults (94).

The lymphatic system. Comparisons of the compositions of the two media

can leave no doubt that lymph is an ultrafiltrate of blood plasma (13, 105, 210). The lymphatics appear to provide an alternative route by which fluids may be returned from the extracellular spaces to the blood stream. They also seem to have the faculty, not possessed by the blood capillaries, of removing from the tissue spaces particulate matter and large molecules such as proteins and lipids. Foreign proteins are not absorbed directly from the tissues into the blood stream, but find their way thither through the lymphatic channels (131, 268) unless the permeability of the blood capillaries has been impaired (132). Even larger particulate materials enter lymph channels from the interstitial spaces with relative ease, although they do not seem to be able to escape in the opposite direction (133, 227, 274). The fact that the concentration of protein is regularly higher in lymph than in the interstitial spaces is further evidence that colloids can enter but not leave the lymphatics. The colloid osmotic pressure of the proteins that gain access to the lymph, aided by the tissue pressure, provides a force by which the lymphatics may be filled. The flow of lymph from the extremities is accelerated by active contraction of the muscles (422, 435) which, aided by the valves in the lymphatic trunks, provides additional propulsive energy. Lymph flow varies directly with the quantity of fluid in the extracellular spaces, presumably because this influences tissue tension and the quantity of protein in the interstitial fluids (209, 422). Filling of the lymphatic capillaries—and, to this extent, the flow in these vessels—is opposed by the force of gravity and by the colloid osmotic pressure in the interstitial fluid about these capillaries (96).

Concerning the absolute volume of the lymphatics there is no information. Both the volume and flow of lymph in the thoracic duct increase when food and fluids are introduced into the gastrointestinal tract, when gastrointestinal secretions are stimulated and when fluids are injected intravenously (421).

Interstitial fluid. From analyses of edema fluids (176, 177, 206, 273) and by direct sampling of extracellular fluid from muscles (290), it has been established that the interstitial fluids, like lymph, have the the characteristics of ultrafiltrates of plasma, differing from plasma only in containing smaller quantities of proteins and lipids. The cations in such fluids consist chiefly of sodium, while the principal anion is chloride, distinguishing them from most intracellular fluids in which potassium and phosphate predominate. The total quantities of sodium and chloride in the body could be accounted for if they were confined to a volume of plasma ultrafiltrate equal to 20 or 30 per cent of the weight of the body (199). After the injection of a number of substances, among them sulfocyanate, sulfate, sucrose (263), magnesium (374) and bromide (49,372,445), the concentrations of these substances in the plasma indicate that they are dispersed through a volume of fluid of the same order of magnitude. The same is true of radioactive sodium (242, 281) and chloride (443). If solutions containing ordinary sodium or chloride are injected, the increments of these ions in the plasma indicate that the added ions are confined to a space of about the same size. Analyses of muscles yield enough chloride and sodium to account for an extracellular volume of about 20 per cent (114, 115, 205, 290), which coincides

with the volume into which these ions will diffuse when muscle is suspended in salt solutions (112, 438).

There is, therefore, a differentiated body of fluid, amounting to from one-fifth to one-third of the body mass, which has the characteristics of an ultrafiltrate of blood plasma with which it is in diffusion equilibrium. This body of fluid contains most of the sodium and chloride in the animal. Individual tissues, on the other hand, contain quantities and proportions of sodium and chloride that cannot be attributed to such an extracellular ultrafiltrate (11, 126, 197, 199, 282, 283). The quantities of these constituents in cells vary from tissue to tissue, being relatively large, for example, in red blood cells, the cells of the gastric and intestinal mucosa and the kidneys (11, 283), almost negligible in muscle and liver cells (11, 126). These intracellular fractions of sodium and chloride do not appear to traverse cell membranes freely. Radioactive sodium penetrates the extracellular fluid with great rapidity, but comes into equilibrium only slowly with the sodium in tissues that contain high concentrations of this element (242, 281). Amberson was able, both *in vivo* (11) and *in vitro* (314) to wash chloride easily from muscle, liver and kidney cells, the concentration of chloride in these tissues when plotted against time, describing a curve that approached zero. From other tissues with initially high chloride it could not be easily removed and the curves of concentration in these tissues approached not zero, but a positive value. Part of the chloride in these tissues was restrained from escaping.

Variation of the concentration of sodium in blood plasma, instead of changing the concentrations of sodium in the blood cells, causes water to move to and from the cells in such a manner that the osmotic pressures in the two media remain always identical (318). Similar reactions between tissue cells and the extracellular fluid have been demonstrated by indirect methods (110, 112, 114, 115, 300, 458). From such experiments it has been estimated that all or almost all of the sodium in extracellular fluid must be osmotically active, that sodium salts are osmotically the most important components of this fluid, that they do not diffuse across cell membranes and therefore control the distribution between the intracellular and extracellular compartments of water, to which all cell membranes are permeable. The impermeability of cells to sodium is not unconditioned: sodium will enter even muscle cells when they become depleted of potassium (219, 220), but the transfer is probably not effected by diffusion alone.

The volume of the extracellular fluid can be estimated only from measurements of the volumes of distribution of a number of substances that diffuse freely from the blood stream through the extracellular fluid, but are more or less completely excluded from cells. At the end of an appropriate interval after administration of the test substance its concentration in the serum and the amount excreted are measured. The volume of distribution, E, is then estimated by the formula $E = \frac{A - U}{S_w}$ in which A = the amount of substance given, U the amount excreted in the urine, and S_w its concentration in the water of blood serum. None of the test substances commonly used is completely excluded from cells. The volume

of distribution of sodium is regularly larger than that of chloride (443) or bromide (424). The distribution of thiocyanate differs from all three. It enters red blood cells, probably certain tissue cells, and fluids in the gastrointestinal tract (81, 263). Sucrose (and probably sulfate) are probably more nearly restricted to the extracellular fluid. Neither enters blood cells nor appears in appreciable quantities in gastrointestinal fluids. On the other hand, both are so rapidly excreted by the kidneys that it is hard to maintain adequate concentrations in the serum for a sufficiently long period, and correction for excretion is undesirably large if renal function is normal (263). In these respects thiocyanate, which is excreted slowly, has an advantage over other test substances which have been proposed. The distribution of test substances is not the same in all species (242, 443).

Estimation of the volume of extracellular fluid from the increments or decrements of serum sodium or chloride, or both, produced by administration of a known amount of sodium chloride, requires the administration of such large quantities of salt in such high concentrations that the osmotic effects of the injected solutions themselves alter the distribution of water in the body. Changes in the volume of the extracellular fluids can be estimated with reasonable accuracy from balances of these ions and their concentrations in serum by the equation, $\Delta E = \frac{B - E_1 (\Delta S)}{S_2}$ in which ΔE = the increase of volume, E_1 = the initial volume, ΔS the increase of concentration in the serum of test material, and S_2 its final concentration in water of serum. This requires that some value for E_1 be assumed.

In eight normal adults by radioactive sodium Kaltreider (242) found extracellular fluid volumes of 20.8 to 28.7 per cent of the body weight, averaging 24.8; while by thiocyanate with correction factors of Lavietes et al. (263) they were 19.5 to 25.8 per cent, averaging 23.3, which agrees with values found by Lavietes (263). By sucrose volumes about 10 per cent lower were obtained by Lavietes: 16.4 to 28.0 per cent, averaging 21.4 per cent of the body weight. The volume in a given individual is far more constant than the volumes in a group of individuals and is characteristic of the individual (263). The ratio of extracellular fluid to body weight is smaller in obese than in thin subjects both in humans (242) and in dogs (199). This ratio also varies from species to species, being nearer 0.30 than 0.20 in dogs (199, 443). The proportion of total water as well as the ratio of extracellular to intracellular fluid diminishes with age from fetal to adult life (232, 457).

Forces that vary the volume of extracellular fluid. Smith and Mendel (362) in 1920 showed that within a few minutes after intravenous injection of quantities of saline or glucose solutions equal to the total blood volume, the blood volume of rabbits had returned practically to its initial level and no gross accumulations of fluid could be found in the animals. Similar observations have been made by numerous investigators (10, 340, 387). Ingested water is treated in much the same way (216, 328, 359, 360). The water is stored, chiefly in the interstitial spaces (205, 261, 387). Presumably the accession of fluid to the blood stream

increases the hydrostatic pressure and diminishes the colloid osmotic pressure enough to accelerate the current from capillaries to interstitial spaces. Processes conducive to dehydration and hemoconcentration, conversely, detract from the volume of the interstitial fluid (115, 141, 294, 300, 310, 458). If the concentration of sodium salts in the body is increased the cells will yield water to the extracellular fluid because, since sodium does not penetrate cells, the effective or electrolyte osmotic pressure of the extracellular fluid rises (115, 318, 444). Vice versa, reduction of the concentration of sodium salts in the extracellular fluids causes the cells to expand at the expense of these fluids (110, 294, 299, 387, 458). There is every reason to believe that alteration of the osmotic pressure of cellular contents causes similar exchanges of water, but reactions of this type cannot be elicited by the mere administration of salts.

Changes of the hydrogen-ion concentration of the body fluids also cause shifts of water between cells and adjacent interstitial fluids, by reactions that have been most precisely defined by Van Slyke (411, 412). When acid is added to a biological medium containing protein the acid anion combines with base derived from the weakly dissociated protein to form a strongly dissociated salt. This increases the number of osmotically active components in solution. Acidification increases the osmotic pressure of cells more than it does that of extracellular fluid because cells contain more protein. It therefore causes water to pass from extracellular fluid into the cells, while alkalinization moves it in the opposite direction. Such exchanges of water, in response to changes of CO_2 tension, have been demonstrated in the blood both in the test tube (187, 218, 411, 412) and in the circulation (104, 218, 321), and in the muscles of living animals (114).

Intracellular fluid and its control. Cellular membranes within the body appear to be generally permeable to water, hydrogen-ions and probably ammonia, but to few other solutes. In most animal forms there seem to be no barriers to the diffusion of urea (113); but in certain fishes uniformity of osmotic pressure is largely maintained by urea, which plays the rôle that in other animals is delegated to electrolytes (4, 366, 368). Glucose is distributed uniformly through the water of plasma and cells in the blood of man (250, 380) and certain other species, but is far less concentrated in the blood cells of rabbits and some other mammals (381). Direct proof that tissue cells admit glucose freely is hard to obtain because the sugar is so rapidly utilized by these cells (77). Solutes that can move unrestrainedly through cell membranes contribute to the total osmotic pressure of all the media to which they have access, but do not affect the distribution of water between media.

The formation of glycogen from glucose enables the cells to store large quantities of sugar in such large molecular form that the carbohydrate is effectually imprisoned in the cells and exerts a minimal osmotic pressure. Lipids, because of their insolubility, must be osmotically almost inert. These intracellular colloids, although they occupy considerable space, must exert a minor influence upon the distribution of water. Protein would occupy a similar position were it not for its ability to combine with a great variety of other substances.

Of chief concern from the standpoint of the internal water exchange is the

disparate distribution of electrolytes. The almost complete exclusion of sodium from cells and the accumulation of potassium within them could not be achieved by processes of diffusion alone; it would require the expenditure of no mean amount of energy. If the metabolic activities of blood cells are reduced to a minimum by chilling, no appreciable quantities of either sodium or potassium enter or leave the cells when the concentrations of these elements in serum are greatly increased or diminished (116, 318). Water passes in or out of the cells in response to such alterations of the concentrations of cations in plasma as if the sodium originally in the plasma and the added sodium or potassium were all osmotically active (116). When blood is placed in the incubator, so that the metabolism of the cells proceeds, potassium moves across the cell membrane, the direction of its motion depending not upon its concentration, but upon the nature of the metabolic activities of the cells (89). Inorganic phosphate manifests the same type of behavior (185). The energy required for these ionic transfers is presumably derived from the metabolic reactions with which their motions are associated. The concentration of Na+K is greater in blood cells than in plasma. Moreover, in the circulating blood the concentration of base in blood cells, unlike that in serum, can vary considerably without proportional transfers of water (116, 320). These and other observations (379) comprise a growing body of evidence that the inorganic components of cells are not all osmotically active as those of plasma appear to be.

The general chemical patterns and behavior of tissue cells are similar to those of blood cells. The cell membranes are not even freely permeable to the inorganic components that are particularly plentiful in cells. Injected magnesium, for example, appears to be confined to the extracellular fluid (374). Inorganic phosphate penetrates muscle cells freely neither *in vitro* (109) nor *in vivo* (326). The volume of distribution of injected phosphate, though somewhat larger than the volume of the extracellular fluids, falls short of the total fluid of the body (312). The volume of distribution of potassium, immediately after its injection, is greater than that of the extracellular fluid, sometimes even exceeding the total fluid of the body. Subsequently it is released from the cells in which it has accumulated, to be excreted by the kidneys (44, 437, 445). Although sodium is usually excluded from muscle cells, it will enter them in animals that have been depleted of potassium (219). Undoubtedly these inorganic materials contribute to the intracellular osmotic pressure, but not as inevitably as do the inorganic constituents of the extracellular fluids. Elkinton, Winkler and Danowski (121) found that, although extracellular fluid changes could be estimated with accuracy from balances of sodium and its concentration in serum, total water changes could not be estimated with the same accuracy from the concentrations and balances of total base or of sodium + potassium. It may be inferred that fractions of potassium, magnesium and phosphates in the cells are not osmotically active and consequently do not control water exchanges, presumably because they form undissociated or partly dissociated combinations with proteins. This permits their concentrations to vary, within limits, in accordance with the activities of the cells, without obliging the cells to imbibe or eject proportional

quantities of water. The passage of electrolytes and probably many organic compounds appears to be linked with metabolic reactions of these cells which provide the energy required to effect their transit.

The volume of the total and intracellular fluids. The volume of the intracellular fluids can be estimated only by subtracting the volume of the extracellular fluid from the total fluid. By weighing animals before and after desiccation Darrow and his associates (199) have measured the total water in a number of species. In rabbits this amounts to about 75 per cent of the body weight; in dogs to about 65 per cent; and in macacus monkeys to about 70 per cent. The proportion of water is greatest in lean, least in adipose animals.

As yet only one substance, heavy water, has been found which distributes itself rapidly and evenly throughout all the water of the body and which is not destroyed nor altered in the body. By means of D_2O, Flexner, Gellhorn and Merrell (139) found 65 per cent of water in guinea pigs; Hevesy and Jacobsen (221) found similar amounts in rabbits. In the guinea pig Flexner et al. (139) estimated that 73 per cent of the water of the blood was exchanged with the extravascular water in the course of a minute. In the rabbit D_2O was distributed throughout the extracellular fluid within 30 seconds and throughout all the water of the body within 30 minutes after its injection.

By means of urea Painter (315) obtained reasonable values for the total water of dogs. Small amounts of this compound may, however, escape recovery and large corrections must be made for its continual production in the body and its rapid excretion by the kidneys. It has been shown that sulfanilamide, which Marshall (285) claimed had the desired properties, as well as other drugs of its group, form compounds with proteins and are most capriciously distributed through blood and tissues (118, 211). Thiourea has been tested by Purple and Lavietes and by Danowski (90). Although originally reported to be inert, universally diffusible and completely excreted (40), a small fraction is stored or destroyed in the body. It appears to be more useful as a measure of changes in the volume than as a measure of the absolute volume of the total water (90).

The total water balance can be estimated with considerable accuracy by the metabolic method which is discussed below. Estimations from balances of sodium and potassium and their concentrations in blood plasma have proved unreliable for reasons mentioned above (121).

EXCHANGES OF WATER WITH THE ENVIRONMENT. *Thirst.* The sensation of thirst does not seem to originate in dryness of the oral mucosa. It is not affected by absence of salivary glands (385) or resection of the olfactory, gustatory or trigeminal nerves (32). Even in diabetes insipidus thirst can be slaked through a stomach tube (138), while a dog with an esophageal fistula will drink enough to meet its immediate needs (7). When their diets and water intakes are varied, dogs will drink in proportion to the water deficits they incur (341). Dill (100) has reported a burro that, after excessive sweating in tropical heat, drank without a pause 12 liters of water, replacing its fluid deficit with considerable accuracy. Drinking is attuned not only to the volume of fluid in the body, but also to the concentration of solutes in this fluid. Gilman (165) has suggested that the stim-

ulus to thirst is the water content of the cells. After intravenous injection of hypertonic sodium chloride, dogs immediately consumed enough water to restore the osmotic pressure of the body fluids to its initial level, despite the fact that they already contained excessive amounts of water. On the other hand, dogs depleted of salt by Darrow and Yannet (95) evinced no thirst despite hemoconcentration. Dogs evinced less thirst after urea solutions than they did after salt solutions of equal osmotic strength (165). The influence of cellular hydration must be less than absolute, since animals or men who have been depleted of salt and water will drink enough water to reduce the concentration of salt in the body fluids below normal, and animals dehydrated by urea do manifest thirst.

Alimentary exchanges of water. In the middle of the last century Bidder and Schmidt (36) demonstrated that enormous quantities of water and salts are continually poured into the stomach and intestines, only to be again reabsorbed. The similarity between these fluids and blood serum and the influence of this exchange upon the internal environment were vividly illustrated by Schmidt's (349) studies of epidemic cholera, dysentery and catharsis. The full implications of these phenomena were recognized by Gamble (150, 152, 153), who showed that all the secretions of the alimentary canal and of the digestive glands, in spite of the diversity of their chemical patterns, have the same osmotic pressure as body fluids. This has been verified not only by chemical analysis, but also by measurements of vapor pressure (167). The osmotic pressure of the gastric (167) and pancreatic (21) secretions varies with that of the serum.

Except in the acid secretions of the stomach, where it is largely replaced by the hydrogen ion, sodium is the predominant cation of these fluids, while the anions consist almost entirely of chloride and bicarbonate (31), unmistakable evidence that the secretions are derived from extracellular fluids.

Solutions introduced into the alimentary canal become isotonic with blood serum during the process of absorption. This adjustment is accomplished by excretion of water and salts and the simultaneous absorption of solutes, if the latter can be absorbed. A hypertonic solution of glucose increases in volume by the addition of salt and water until it reaches the osmotic pressure of serum. A hypotonic solution loses volume, although it gains salt, until it reaches the same pressure. But even while these solutions are gaining or losing fluid, glucose is being withdrawn from them into the blood (146, 275, 330). The actual quantity of fluid excreted in response to the ingestion of food or fluid is determined by the osmotic pressure of the ingesta. The amount of water ultimately left in the normal gut depends upon the quantity of solutes which is not or cannot be absorbed. Water or solutions containing completely absorbable solutes may be entirely absorbed, but before this process is terminated the solution is replaced by a salt solution having a composition characteristic of the secretions of the gut (55). If an ion, like sulfate, which cannot be easily absorbed, is introduced, little salt enters the gut and what does enter is eventually almost completely reabsorbed, leaving an isotonic solution of sulfate salt (97, 228, 416). In the stomach secretion follows the same principles as it does in the intestine; but absorption (75, 117), even of water (74), is almost negligible. In

the colon absorption predominates; this is the site of the final concentrating process that yields the semisolid feces (12, 171).

The alimentary canal affords little protection to the internal environment. Absorption of water and salt does not cease when there is a plethora of these materials in the body. Secretory activity continues when the salt concentration and volume of the body fluids is depleted (248, 269, 378). Since these secretions are reabsorbed this has no vicious effect so long as the alimentary canal, kidneys and other organs are functioning efficiently. If, however, the gastrointestinal contents are removed by vomiting, diarrhea, by a tube or through a fistula, the uncontrolled secretory activity may lead to disastrous dehydration and salt depletion.

The mass movements of fluid involved in the processes of secretion and reabsorption require some other force than osmotic pressure. Wells (427, 428, 429) believes these movements are controlled by a balance between the colloid osmotic pressure of the proteins in the tissue fluids of the intestinal villi which tends to withdraw fluid from the intestine, and the hydrostatic pressure on the two sides of the intestinal mucosa. This equilibrium is defined by the equation $\frac{dw}{dt} = K(TP - OP_t - IP)$, in which $\frac{dw}{dt}$ = the rate of passage of fluid into the intestine, TP = the hydrostatic pressure in the tissue spaces of the villi, OP_t = the colloid osmotic pressure of the fluid in these spaces, and IP = the intraintestinal pressure. TP is a product of the state of congestion of the blood vessels in the intestinal wall and the distention of the lacteals. Wells found that the introduction into the intestines of food and fluid regularly evoked circulatory congestion, which disappeared when absorption began (430). He has estimated that the magnitude of the various forces involved meets the requirements of the equation and has shown by a large series of experiments that the direction and rate of flow of fluid across the intestinal wall vary with TP and IP as theory demands (429).

Loses of water through skin and respiratory passages. The insensible perspiration, water constantly lost by evaporation from skin and respiratory passages, must depend upon the differences in temperature and water vapor pressure between the surface from which evaporation takes place and the environmental atmosphere. During normal respiration, with the atmospheric temperature between 23° and 25°C. the aqueous tension of alveolar air is about 2 mm. Hg lower than it would be if the air were completely saturated at rectal temperature. As the environmental temperature rises to 37.5° to 41° the vapor tension of the alveolar air also rises. Saturation is more nearly attained during a period of apnea; alveolar vapor pressure falls during hyperventilation (68). The insensible water from the skin seems not to emerge through the skin to evaporate on the surface, but to escape as water vapor directly from within the skin, which is permeable to gases, but not to water (289). So long as the sweat glands are inactive the insensible perspiration is almost, if not altogether, devoid of solutes (33, 147, 188, 413, 415).

Metabolic measurement of water balances. Vaporization from skin and respiratory passages is one of the chief processes by which heat is eliminated from the body. When heat production is moderate and when environmental temperature and humidity are equable, approximately 25 per cent of the heat produced by a normal individual is lost by evaporation. Newburgh (234, 311) proposed that this relation be used to estimate water balances in the following manner. The water balance, WB, may be defined by the equation, $WB = (W_2 - W_1) + (S_e - S_f) + (C + 0.54P + F)$, in which W_2 and W_1 are the final and initial body weights, S_e and S_f are the solids of excreta and ingesta, while C, P and F are the weights of carbohydrate, protein and fat burned by the subject. The first two terms, the weight gained and the solids lost, may be measured directly with considerable accuracy. The last term, the quantity of food burned, can be estimated only by indirect methods that involve a series of assumptions: that C is equal to the carbohydrate eaten, that P can be calculated from the nitrogen excreted in the urine, and that the insensible loss bears a fixed relation to the heat production (for details of methods of calculation see (262, 324)).

With atmospheric conditions accurately controlled in normal subjects under basal conditions, insensible loss is a reasonably accurate measure of heat production. Any change of body temperature, since it signifies that heat loss and heat production are not equal, introduces an error. Exercise—even such slight exercise as shivering—may disturb the normal relations between evaporation and heat production. At temperate or high temperatures the burden of heat dissipation imposed by exercise falls entirely upon evaporation (106, 107, 192). Sensible perspiration invalidates the procedure because, when sweating begins, the proportion of heat lost by evaporation mounts rapidly (106, 450). Moreover, water lost from the skin as sweat may escape evaporation. Nevertheless, in normal subjects, if environmental temperature and humidity and physical activities are kept within such limits that sweating and shivering are eliminated, the relation of vaporization to heat production is remarkably constant (234, 265, 311). In a series of studies by Newburgh et al. (311) heat production, estimated from insensible loss, the carbohydrate of the diet and urinary nitrogen, differed by less than 5 per cent from indirect calorimetric measurements when averages of several 24-hour periods were taken. Greater discrepancies were encountered in shorter periods of observation (234, 311).

In febrile diseases the method cannot be used. In certain other diseases and disorders the proportion of heat lost by vaporization may be abnormal (86, 164, 237, 262, 377). In hyperthyroidism vaporization appears to be relatively increased, probably because sweating is seldom completely abolished (86, 237). In myxedema an unusually small proportion of heat is lost by evaporation (164). A number of observers have detected no changes of insensible perspiration after oral or parenteral administration of moderate (69, 239, 266, 267) or even considerable (184) amounts of water or isotonic salt solution. Patients with established edema appear to have normal insensible losses (238, 249, 377). On the other hand, the simultaneous administration of excessive amounts of water and pituitrin, which greatly increases the load of water in the body, seems

to exaggerate vaporization (184, 280, 339), while severe dehydration diminishes it (5, 184, 280, 444). Injection of hypertonic salt solution also decreases it (170). Gilman and Barbour (166) reported that insensible losses vary with the osmotic pressure of the body fluids. Although osmotic pressure must influence vaporization the large variations of insensible loss noted by Gilman and Barbour cannot have been referable directly to changes of osmotic pressure, since the difference between the vapor pressures of blood and distilled water amounts to only about 0.3 per cent of the total vapor pressure of such solutions at body temperature.

Sensible perspiration (*sweating*). When the environmental temperature, humidity or heat production rises so high that the necessary amount of heat cannot be eliminated by the usual processes, the sweat glands are brought into play. In the nude resting subject at moderate humidity sweating becomes perceptible at about 31°C., but clothing or even slight muscular activity will bring it on at 25° to 28° (106, 191, 194, 450). When the temperature exceeds about 35° the heat lost by evaporation exceeds the heat produced, because radiation no longer eliminates heat from, but contributes it to, the body (106, 449). Subjects studied by Adolph and Dill (6, 8) at Boulder City, at a mean shade temperature of 35.3°C. drank an average of 4000 cc. of water daily, although they drank only 1400 cc. in Boston during the winter. Voluntary intakes as high as 13,600 cc. of water per day have been recorded. During exercise one subject eliminated 1700 cc. of water in an hour. Respiratory losses of water increase only slightly with temperature in man, since humans do not augment the respiratory minute volume appreciably in behalf of temperature regulation (6). The dog, lacking sweat glands, resorts to panting to eliminate excessive quantities of heat. Since this mechanism is less efficient than sweating, the dog adapts less effectively than man to extremely hot atmospheric conditions (101).

Women have the ability, not shared by men, to reduce heat production at moderately high temperatures, thereby delaying the onset of sweating (193). Persons who are partially or totally deficient of sweat glands are unable to prevent the body temperature from rising in hot climates, especially when they exercise (140, 338); their insensible loss is, however, quite normal (338). During the first few days of sojourn in a hot climate the quantity of sweat produced in response to a given stimulus increases, while the stimulus required to initiate sweating and the interval between stimulus and response diminish (448, 449). This is part of the process of acclimatization to the tropics.

Both insensible perspiration and the secretion of sweat appear to be regulated by the amount of blood circulating in the skin (194, 449). The controlling factor cannot be the temperature of the skin itself, because this may diminish as a result of evaporation if sweating is profuse. Winslow and Gagge (448) have suggested that sweating may be controlled by the internal (rectal) temperature, which increases slightly under conditions that promote sweating, even when the skin temperature falls. A rise of internal temperature, even without change of environmental temperature or humidity, will induce sweating.

Sensible perspiration differs from insensible in carrying with it solutes, among them electrolytes, chiefly sodium and chloride (102, 103, 136, 188, 225, 294, 295, 306). This marks its origin from extracellular fluids. As the rate of sweating increases the concentrations of sodium and chloride rise (102, 188). The ratio of chloride to sodium in sweat is not, as it is in extracellular fluids, about 1.4:1.0, but approximately 1:1 (102, 295). Sweat is distinctly hypotonic, the average concentrations of Na and Cl in the sweat of males being only about 30 mM. per liter, varying from 10 to 70 mM. (102, 295). Some persons habitually lose more sodium and chloride per unit of water than others (102, 295) without relation to the concentrations of these ions in their sera (102). Women regularly lose less than men do (295).

As sojourn in a hot climate is prolonged the salt content of sweat diminishes (73, 102, 295). The capacity to adapt in this manner to prolonged or frequent exposure to heat varies from individual to individual (102, 295). It is relatively easy to deplete some persons of salt by repeated sweating; others, especially women, suffer little depletion despite strenuous measures (295). There is a suggestion in McCance's (294) experiments that the reduction of salt in sweat during adaptation to heat may depend upon the development of a certain degree of persistent salt depletion, since it could be prevented by administration of abundant quantities of salt. Forbes, Dill and Hall (141) noted that the average volume of interstitial fluid of men fell somewhat when they moved to a hot climate, an indication that their bodies contained less sodium and chloride. Sweating *per se* tends to withdraw more water than salt from the body; but it does, in the unacclimatized subject, cause salt depletion. If only water is taken to replace the lost fluid the concentrations of sodium and chloride in extracellular fluids fall (225, 306).

Perspiration, insensible and sensible, is attuned to the regulation of temperature rather than body water. Insensible loss does diminish slightly in severe dehydration and when the concentrations of sodium and chloride in extracellular fluid rise inordinately. Men exposed to high temperatures sweat less when deprived of water than when they are allowed to drink freely (178). Their temperatures consequently rise. There is no evidence that sensible perspiration is promoted by drinking unnecessarily large amounts of water (8).

Measurement of water balances in sweating subjects offers peculiar difficulties since water of vaporization no longer bears its usual relation to heat production. In a large proportion of conditions, especially when sweating is profuse, an error in the evaluation of fat burned, which is the factor derived from insensible loss, is of relatively little significance in the calculation of the water balance. Errors that may arise from changes of body temperature are also comparatively small. Estimation of water balances from balances and concentrations in serum of sodium and chloride are totally invalidated by sweating.

REGULATION OF BODY FLUIDS BY THE KIDNEYS. Chief responsibility for maintenance of the volume and composition of the body fluids devolves upon the kidneys. The elimination of abnormal substances and superfluous amounts of normal constituents is no more important than the conservation in proper

proportions of essential components, including water. There can no longer be reasonable doubt that these objects are achieved by a combination of filtration, reabsorption and secretion. Ingenious methods for the measurement of these three processes in the intact animal, all depending upon the clearance principle, have been devised, chiefly, by Smith (367) and his associates.

The term *clearance*, originally introduced by Van Slyke (17), represents the virtual volume of plasma cleared of a given solute in a standard time. Clearance $= \frac{UV}{P}$, in which V is the rate of urine flow, while U and P represent the concentration of the test solute in urine and plasma respectively. The clearance of any substance that is completely extracted from the blood plasma in its passage through the kidney measures the flow of plasma through the kidney. A substance that has the properties required for measurement of renal plasma flow has been found in diodrast (370). The clearance of any substance that passes the glomerular filter freely, but is neither reabsorbed nor secreted by the tubules, will equal the rate of glomerular filtration. In man and most animals inulin (334, 367), a number of hexitols such as mannitol, sorbitol and dulcitol (375), and sucrose (368) have the properties required for measurement of filtration. In the dog creatinine, first proposed for man by Rehberg (332), may also be used. The difference between the filtration rate and the rate of excretion of the fully elaborated urine gives the rate of reabsorption of water. The rate of secretion or reabsorption of any solute may also be measured by comparing its clearance with that of one of the purely filtrable solutes.

By these methods Smith (368) has estimated that the average renal blood flow of an adult male amounts to about 1200 cc. per minute, plasma flow about 700 cc. per minute. The average rate of glomerular filtration is about 130 cc. per minute, the blood plasma losing more than 15 per cent of its water in its passage through the glomerular capillaries. Since the normal rate of urine flow is from 0.7 to 1.5 cc. per minute, 99 per cent or more of the water filtered through the glomeruli is ordinarily reabsorbed in the renal tubules.

In amphibia and other lower forms of life the osmotic pressure of the urine never exceeds that of the blood. The tubules of the mammalian kidney, by acquisition of the loop of Henle and the terminal segments of the renal tubules (383), can reabsorb water against the force of osmotic pressure, to produce a urine with an osmotic pressure far above that of plasma. It has been shown by direct analysis that in amphibia (419) and in mammals (418), in its passage through the proximal tubules glomerular fluid loses all its reducing substances and a large proportion of its water and salt, leaving a solution with an osmotic pressure equal to that of the blood, but with a greatly altered pattern. Shannon (354) has suggested that in the proximal tubules glucose and other constituents with 80 per cent of the filtered water are withdrawn by a process of obligatory reabsorption; in the loop of Henle and the distal convoluted tubules electrolytes and water are further reabsorbed to establish the ultimate concentration and reaction of the urine by a facultative process.

In the mammal the concentrating power of the kidney—i.e., the power to

reabsorb water against osmotic pressure—does not seem to be a single function limited specifically by osmotic pressure. It has been shown by several observers (3, 154, 155, 169) that the limiting osmolar concentration for urea is greater than the limiting concentration for sodium chloride and that the addition of either one does not limit the concentration of the other. Urea and sodium chloride do not compete with one another for water. On the other hand, the concentrations of chloride, bicarbonate, sodium, potassium, inorganic phosphate, glucose and creatinine seem to be limited by the aggregate osmolar concentrations of these constituents, which do, therefore, compete with one another for water (154, 155). The excretion of water, then, depends not only upon the quantity, but also upon the nature of the solutes requiring elimination.

Urea is always more concentrated in urine than in plasma, its clearance at mean rates of urine flow being about one-half as great as that of inulin. In profuse diuresis the clearance rises slightly, in extreme oliguria it falls, the concentration ratio $\frac{U}{P}$ approaching an asymptote. Since the capacity of the kidney to reabsorb urea is limited, the quantity of water preempted by urea for the urine is determined by the quantity of urea filtered which, in turn, must depend upon the rate of protein catabolism. The diuretic effect of urea does not begin only after maximal concentrations have been reached; water and urea are proportioned to one another at all rates of excretion of urea (154, 155). In studies of water deprivation by Winkler, Danowski, Elkinton and Peters (442), the excretion of water by dogs and men varied directly with protein catabolism, although urinary concentrations of nitrogen never reached maximal values. Nevertheless, as water becomes more precious less and less is yielded for each increment of urea up to the limiting concentration. Urea clearances fall, accounting for the nonprotein nitrogen retention of dehydration. As the quantities of urea requiring excretion diminish the volume of urine does not fall proportionally because the influence of other solutes persists. For this reason it is impossible to obtain urine of maximal concentration from subjects with low protein catabolism by restriction of water (304).

Always more water than urea is absorbed; but sodium, chloride and water can be reabsorbed in the most variable proportions in a manner that seems, on the whole, contrived to preserve the volume and electrolyte patterns of the body fluids. From experiments of Walker, Bott et al. (418) 80 per cent of the water and about 60 per cent of the chloride of glomerular filtrate appear to be reabsorbed in the proximal tubules; in the loop of Henle further salt is withdrawn; the final abstraction of water is accomplished in the distal convoluted tubules. Shannon (355) has suggested that the dilute urine of diabetes insipidus may be regarded as filtrate which has been subjected to all these processes except this final abstraction of water. If a large amount of salt remains unabsorbed the power to reabsorb water is limited accordingly; but water may be excreted with little or no salt if the terminal concentrating process is inhibited.

If large amounts of water are given without salt the resulting aqueous diuresis may carry with it from the body a certain amount of sodium and chloride (87,

388); but chloride excretion may even diminish slightly after moderate amounts of water (111). Since ingestion of water tends to lower the concentration of salt in serum and in glomerular filtrate salt should be more completely reabsorbed unless sufficient water were given to increase glomerular filtration (353).

If salt is eliminated from the diet or if the salt content of the extracellular fluids is depleted by sweating or vomiting, urinary chloride excretion promptly diminishes to the vanishing point. A given quantity of water is excreted more slowly by an animal or man who has been depleted of salt than by a normal animal or man (296, 439). This delay in excretion may arise from several factors: 1, circulatory depression that regularly attends salt depletion (310); 2, diminished glomerular filtration that may depend upon the circulatory depression (296, 439); 3, relative or absolute reduction of the volume of the extracellular fluids. The last results from the general tendency not to retain water without a proportional amount of salt and the transfer of water from the extracellular fluids to the cells because of the reduced osmotic pressure of the former.

Excessive amounts of salt or hypertonic salt solution provoke diuresis that may lead to dehydration (240, 444). As the concentrations of sodium and chloride in the serum rise, not only does the excretion of salt increase, but also its reabsorption, albeit to a lesser degree. The concentrations of sodium and chloride in the serum consequently remain elevated or even increase (120, 196, 444, 456). This retention of salt has a double advantage. Displacement of salt from the urine permits other substances that compete with salt to be excreted with greater economy of water. At the same time the accumulation of sodium and chloride in the body, by raising the osmotic pressure of the extracellular fluids, withdraws water from the cells, which thereby share in the dehydration which would otherwise be borne entirely by the extracellular fluids.

This reaction is observed not only after excessive doses of sodium chloride, but in most states of dehydration that do not originate from primary salt depletion. When either dogs (119) or men (442) are deprived of water, as dehydration becomes advanced, sodium and chloride in the urine fall to a minimum, while their concentrations in the serum rise. If urea is given with large quantities of water enough sodium chloride may be washed out in the diuresis to lower the concentration of serum chloride (276). If dehydration is induced by giving urea with inadequate amounts of water, the concentrations of sodium and chloride in the serum rise (120). When large quantities of sodium sulfate are injected into dogs chloride mounts rapidly (351). Addition of sodium chloride to the sodium sulfate does not increase chloride excretion, but only accelerates the elimination of the sulfate, raising the concentrations of sodium and chloride in the serum still further. Inorganic phosphate tends in the same manner to displace chloride from the urine (304, 446).

Potassium is eliminated at a somewhat faster rate than sodium and, instead of suppressing the excretion of the latter, causes a transitory sodium diuresis (351, 446). The reason for this may be that a large proportion of any surplus potassium is immediately taken up by cells, leaving its associated ion to combine with sodium in the extracellular fluids.

Glucose which escapes reabsorption in the tubules requires for its excretion the same amount of water required by an osmotically equivalent quantity of salt (154, 155). It does not, however, tend to displace sodium and chloride from the urine. On the contrary it accelerates their excretion to such an extent that it may deplete the body fluids of salt (15, 196, 247). Glycosuria is, therefore, peculiarly dehydrating.

The final concentration of the urine depends upon the quantities in the glomerular filtrate of two groups of components which can be concentrated independently. Maximal economy of water can be attained only if the two are excreted in optimal proportions. One of these, of which urea is representative, can be reabsorbed to a limited extent, but is always more concentrated in urine than in blood plasma. The quantity of water which it withdraws is, therefore, determined almost entirely by its concentration in the body fluids and the rate of glomerular filtration; it can be little influenced by tubular activity. The other group is composed of a large number of solutes which resist the reabsorption of water in proportion to their aggregate osmolar concentration. The only representatives that usually appear in large enough quantities to have an important effect upon the reabsorption of water are the inorganic ions of which sodium and chloride form the bulk. The effect of these two on the volume and concentration of urine is not obligatory like that of urea, because they can be reabsorbed almost completely even when their concentrations in blood plasma are abnormally high. They yield their places in the urine when there is need for conservation of water, either because there is a direct deficiency of this commodity or because it is needed for the elimination of other substances. This permits these other substances to be excreted more rapidly and with greater immediate economy of water. It also provides extra water for their excretion by extracting it from the cells. Concentration, therefore, is not equally affected by all solutes, nor can it be regarded entirely from the standpoint of the ultimate composition of the urine; it must be viewed also in relation to the internal environment.

Species differ in their ability to reabsorb water from the renal tubules against the osmotic pressure of solutes. In this respect the kidney of the dog is far more efficient than that of man (208).

Nervous control of urine formation. Marshall and Kolls (286, 287) demonstrated that injection of adrenalin and stimulation of the splanchnic nerves caused oliguria, while section of the splanchnic nerves and denervation of the kidneys induced diuresis. The effects of splanchnotomy were neutralized by partial occlusion of the renal artery (288). When Richards and Plant (335, 336) maintained a constant flow of blood through the kidney, adrenalin and splanchnic stimulation had a diuretic effect. The nerves of the kidney, therefore, appear to exert their principal action upon the renal circulation. The negative results of Rhoads, Van Slyke et al. (333) must be discounted because the animals used had been subjected to unilateral nephrectomy. From investigations of the action of adrenalin, theophylline, artificial fever and spinal anesthesia on renal function of normal men (63, 369, 370, 371) by clearance techniques, Smith (369) concluded that filtration is regulated almost entirely by reactions

of the efferent arterioles. By this arrangement filtration could be kept relatively constant in the face of changes of blood flow and blood pressure; reduction of blood flow by constriction of the efferent artery would be compensated automatically by an increase of filtration pressure. From a re-evaluation of Smith's data and calculations, Lamport (256, 257) has concluded that glomerular filtration is controlled by activity of both afferent and efferent arteries, which seems more in keeping with other observations in the literature.

Adrenalin and splanchnic stimulation do not affect the excretion of all solutes equally and therefore presumably affect reabsorption as well as filtration (286, 287). The completely isolated kidney excretes a urine in which all the components are extremely diluted (27, 28, 383). Its loss of concentrating power is chiefly referable, not to lack of nervous control, but to absence of the posterior pituitary hormone (27, 383, 414). The renal nerves may, however, be implicated in the action of this hormone. Bayliss and Fee (27) found that the polyuria characteristic of the isolated heart-lung-kidney preparation did not develop, if the kidney was perfused *in situ* with a heart-lung preparation from another animal, until the nerves to the kidney had been cut. Posterior pituitary preparations do not check, but may even provoke, diuresis in animals anesthetized by ether, urethane and a number of other drugs (9, 52, 80, 305). Bayliss and Brown (26) found that ether anesthesia did not affect an explanted kidney, though it inhibited in a normal kidney polyuria induced by decerebration or hypophysectomy. Anesthetics, therefore, appear to prevent variations in the concentration of the urine by acting through the nerves of the kidney (214).

The oliguria induced by emotional disturbances must probably be ascribed to posterior pituitary, not nervous, activity since it cannot be abolished by denervation of the kidneys and elimination of adrenal medullary activity (345).

THE ACTION OF ENDOCRINE GLANDS. *The hypophysis.* It was long recognized that diabetes insipidus could be produced less consistently by removal of the hypophysis (243) than by injury to adjacent portions of the brain (18, 45, 84, 243). Nevertheless, whatever its origin, the polyuria could be abolished by injection of extracts of the posterior lobe of the pituitary gland (60, 67, 307, 339, 425, 426). This paradox has been explained by two discoveries: first, that the diuretic effect of removal of the posterior lobe of the hypophysis depends upon the preservation of some anterior lobe substance (392); secondly, that the secretory activity of the posterior lobe is controlled by the supraoptic nuclei in the hypothalamus (137, 230, 231, 278). Destruction of these nuclei or the tracts that connect them with the posterior lobe of the pituitary causes diabetes insipidus by inhibiting the secretory activity of this lobe.

The antidiuretic activity of the posterior lobe appears to reside in or with the pressor principle, pitressin (224, 339). According to Fraser (144) the oxytocic principle has a diuretic action in the rat. Heller (214) has succeeded in freeing the antidiuretic factor of pressor activity. Because diabetes insipidus develops only if there is some active anterior lobe tissue, it has been suggested that this lobe produces a diuretic hormone (22, 35, 433). The posterior pituitary cannot be merely an inhibitor of the anterior lobe since it acts upon the isolated kidney.

Biasotti (35) believes the diuretic principle of the anterior lobe is identical with the thyrotropic principle. Schweizer, Gaunt et al. (352) attribute the absence of polyuria after hypophysectomy to the failure of pituitaryless animals to eat. Winter, Sattler and Ingram (455) have shown that experimental diabetes insipidus of animals is minimized or abolished by starvation. Schweizer and her associates found that at the cessation of the initial self-terminative polyuria that followed hypophysectomy the experimental animals ate almost no food and lost weight. When they were given anterior lobe extracts appetite and polyuria returned simultaneously.

Although the posterior pituitaries of all vertebrates, including birds, amphibians, and teleost and elasmobranch fishes, possess antidiuretic principles (214), only the mammalian kidney reacts to these (54, 214). For this reason it has been inferred that it promotes reabsorption of water in the loop of Henle and the terminal segments of the renal tubules, which first appear in mammals. In frogs posterior pituitary extracts have effects of a different nature (46, 47, 214). The antidiuretic principle does not interfere with absorption of fluid from the gut nor its distribution in the body (216, 360). Insensible loss increases when pituitrin is given together with large amounts of fluid; but this probably derives from the overhydration rather than the effects of pituitrin *per se* (216, 280).

The primary defect in diabetes insipidus appears to be polyuria, not polydipsia, since in this condition thirst is abolished by nephrectomy (389, 390) and since water deprivation causes dehydration (138, 355). In hypophysectomized dogs (434) and patients with untreated diabetes insipidus (441) greatly reduced diodrast clearances have been reported. These Winer (441) attributes to disturbance of the secretory activity of the tubular cells rather than diminution of renal blood flow. Glomerular filtration is not altered in diabetes insipidus (59, 327, 355, 434). The transitory reductions of renal blood flow (189, 434) and glomerular filtration (434) immediately after injections of pituitrin are probably referable to vasopressor action of this extract. Its antidiuretic action persists after blood flow and filtration have again become normal (26, 356, 434). The most characteristic disorder of diabetes insipidus, experimental or spontaneous, is a defect in the tubular reabsorption of water that leads to the passage of large amounts of extremely dilute urine (54, 189, 325, 358). The dehydration is constantly combatted by ingestion of large quantities of water. Since both drinking and urination are intermittent functions the concentrations of solutes in the blood plasma are unusually variable. After administration of water the conductivity of the serum falls as it does in normals (135). After complete withdrawal of fluid (355) or administration of salt (70, 196, 355) serum sodium and chloride rise more rapidly than usual. This has given rise to the opinion that failure to reabsorb water is attended by excessive reabsorption of salt. The responses of the animal with diabetes insipidus to administration or deprivation of water are qualitatively similar to those of a normal animal, but are exaggerated by the extreme compulsion. The animal is in a continuous state of mild water depletion because drinking tends to fall somewhat behind urination. At the onset of experimental diabetes insipidus considerable sodium and chloride are

excreted before equilibrium is established (454). Although the concentrations of sodium and chloride in the serum rise quite early if water is completely withdrawn, some salt is lost in the urine by the continuing diuresis (355). Urea clearances are high in proportion to creatinine clearances in diabetes insipidus just as they are when the rate of urine formation is greatly accelerated by the administration of water (59, 233, 327). When the water intake is limited the volume of urine does not fall with the usual rapidity nor does its concentration rise to the usual extent (355). Dehydration acquires a greater intensity earlier. After only 24 hours of water deprivation a dog with diabetes insipidus, studied by Shannon (355), had lost about 40 per cent of its body water and its serum sodium had risen from 145.3 to 175.5 mM. per liter, a degree of dehydration that it would have required several days to produce in a normal dog. The polyuria of diabetes insipidus is exaggerated by sodium chloride; but the concentrations of sodium and chloride in the urine do not rise so high, while those in serum rise higher than usual (70, 264, 355). Chu, Lin and Yu (70) found that the administration of salt increased the excretion of potassium and nitrogen, a response noted by Winkler, Elkinton et al. (444) when the concentrations of sodium and chloride in the extracellular fluids rose excessively. Hare, Hare and Phillips (196) have shown that the ratio, concentration of chloride in reabsorbed fluid: concentration of chloride in serum, does not fall after administration of hypertonic salt solution in diabetes insipidus as it normally does. This does not mean that sodium and chloride are more completely reabsorbed, but that the salt that is not reabsorbed demands more water for its excretion.

The degree of polyuria also varies directly as the nitrogen excretion (453); the kidneys in diabetes insipidus can concentrate urea no better than they can salt. When the polyuria is checked by starvation the specific gravity of the urine remains low. The relative oliguria can be attributed to reduction of the solutes requiring excretion. The concentration of urine will rise, but not to the normal degree under the influence of extreme dehydration (355).

When active posterior pituitary extracts are given to animals or persons with diabetes insipidus the flow of urine falls sharply, while its concentration rises; the excretion of sodium and chloride diminishes (264, 363, 426, 454) and the urea clearance falls (59, 233, 327, 355). Equilibrium is, however, established after a short interval (454). When pituitrin is given to a normally hydrated or a dehydrated animal the rate of urine formation is not appreciably altered (9, 52, 88, 307, 325, 339). When it is given with a dose of water the diuresis which would ordinarily result is abated or suppressed. The effect of a given dose of pituitrin is inversely proportional to the amount of water that is given (325). It is doubtful whether the antidiuretic hormone actually accelerates the excretion of salt as some claim (358, 363, 408, 454); it does seem to check the excretion of water without altering the rate of elimination of sodium and chloride (88, 339). It therefore exaggerates the hemodilution that normally follows the ingestion of water (9, 52, 339, 360). Its antidiuretic action varies directly as the amount of water and inversely as the amount of salt given (9, 52, 80, 298, 307). Melville (301) claims that posterior pituitary preparations have a positive diuretic effect

on animals which have been loaded with salt. When, however, Adolph and Ericson (9) gave pituitrin with varying amounts of water, but a constant dose of salt, the volume of urine excreted was always the same. Enough water was eliminated in every instance to permit excretion of the salt in less than maximal concentration; the remainder was reabsorbed under the influence of the pituitrin.

Gilman and Goodman (168) demonstrated in the urine of dehydrated rats material resembling the antidiuretic principle of the posterior lobe. They suggested that secretory activity of the posterior pituitary is automatically regulated in conformity with the water content of the animal. Although this observation was abundantly confirmed (186, 213, 231, 417), antidiuretic material was not found in the urine of other mammals and the identity of the material in rat urine with posterior pituitary hormone was challenged (186, 417). The demonstration of antidiuretic activity in the urine of dehydrated dogs (195) and cats (231), its absence from the urine of dogs (195) and cats (231) with diabetes insipidus, and its presence in the urine of animals that have received injections of posterior pituitary extracts (215, 231, 236) have vindicated Gilman and Goodman's theory.

The suprarenal glands. Adrenalin, according to Kaltreider et al. (241) reduces the volume of the plasma, but increases the volume of cells in the blood of intact animals. Large doses diminish the blood flow through the kidneys and urine excretion (286, 287, 407). Small doses induce diuresis (337, 407), presumably by constriction of the efferent arterioles. Smith (63, 368), because adrenalin reduced renal blood flow and urine formation without altering glomerular filtration, concluded that its action is always confined to constriction of the efferent arterioles. If this inference, which has been challenged by Lamport (256, 257), is correct, the oliguria after large doses must result from excessive tubular reabsorption of water.

As early as 1927 Baumann reported that the sodium and chloride in the serum of animals diminished after suprarenalectomy (25) and that the lives of such animals could be prolonged by injections of sodium salts (284). Loeb (271, 272) showed that the sodium of the serum of adrenalectomized animals and patients with Addison's disease was regularly low and that the symptoms of adrenal cortical insufficiency could be relieved or prevented for considerable periods by administration of large amounts of sodium chloride or sodium bicarbonate. Removal of the adrenals is succeeded by a diuresis in which more sodium than water is sacrificed (201, 272). Swingle and associates (393) first called attention to hemoconcentration. Fluid escapes from the blood stream, not only through the kidneys, but also through the capillary walls. This transudation precedes the obvious symptoms and signs of circulatory collapse that characterize the fully developed syndrome of cortical failure (201, 393). This transudation must be due to circulatory failure. For this the sodium deficit cannot be entirely responsible because cortical extracts will alleviate symptoms and signs of adrenal insufficiency before any reversal of the electrolyte disturbance or even the disposition of fluid in the body can be detected. Adrenalectomized dogs are peculiarly susceptible to water intoxication (393). Patients in the crises of Addison's disease will develop edema if they are given parenteral fluids without

cortical extract (320). Reduction of the concentration of sodium in the extracellular fluid causes the cells to imbibe water (309). Retention of potassium, which accumulates in the cells, should aggravate this tendency; but Harrison and Darrow (198) could detect no increases of cellular water in behalf of potassium, suggesting that this element is retained in an osmotically inactive form.

The chief effect of adrenalectomy upon the kidney is to diminish the reabsorption of sodium. Chloride of serum usually falls proportionately; but in some cases of Addison's disease bicarbonate suffers instead (320). In the initial diuresis which immediately follows adrenalectomy more salt than water is lost. The excretion of water may signify that the general tendency to maintain the electrolyte osmotic pressure of the body fluids is not altogether abrogated. The ability to reabsorb sodium is only impaired, not abolished. Willson and Sunderman (440) found that the concentrations of sodium and chloride in the urine of a patient with Addison's disease did not increase to the usual degree after restriction of fluid. Instead the sodium and chloride of the serum rose and the patient became dehydrated. In advanced renal insufficiency with circulatory failure glomerular filtration diminishes and blood urea rises (396). Ultimately there may be complete anuria.

Administration of cortical extracts completely reverses the disorders of adrenal insufficiency. With free access to food, water and salt, serum electrolytes, body fluids, renal function and circulation become quite normal. Sodium is retained while potassium is excreted. If insufficient salt and water are given some body water is sacrificed with the potassium to restore the concentration of sodium in the extracellular fluids (200). Water diuresis may, therefore, attend both the onset and the termination of adrenal insufficiency.

Adrenal cortical extracts and corticosterone have little effect on the metabolism of water and electrolytes in normal animals (130, 202, 203, 403, 404). Acute retention of sodium after large doses of extract has been reported (202, 203, 404), but not sustained hypernatronemia. Conditions may be devised in which the effect of cortical extract would be evident, just as the antidiuretic action of the posterior pituitary extract is evident only after the administration of water. The protective action of cortical extract against water intoxication (159, 393) may represent such a set up.

Desoxycorticosterone has proved a valuable therapeutic substitute for the adrenal cortical hormone in the treatment of Addison's disease, although its action is confined almost entirely to the restraint of sodium wastage (386, 402, 405). It also has distinct effects on normal animals and, in large doses, has deleterious effects on adrenalectomized animals and patients with Addison's disease. Ferrebee et al. (129) reported that excessive doses of desoxycorticosterone acetate, when given to adrenalectomized dogs or patients with Addison's disease, provoked retention of salt, sometimes sufficient to cause edema, together with hypertension and signs and symptoms of congestive heart failure. These disorders do not arise only from augmented tubular reabsorption of sodium, but also from wastage of potassium which leads to degeneration of the heart muscle (92, 93). They are aggravated by low potassium diets and can be mitigated or prevented

by administration of extra potassium (130, 329). In normal animals desoxycorticosterone provokes profuse diuresis with dilute urine (329).

If it be assumed that the action of desoxycorticosterone on the kidney is identical with that of the natural cortical hormone, all the phenomena that have been described could be explained on the hypothesis that the adrenal cortical hormone promotes reabsorption of sodium, while the posterior pituitary hormone promotes reabsorption of water. It is not necessary to suppose that the actions of these two hormones are antithetical. Since thirst is the natural response to accumulation of salt in the body, polydyspia should follow reabsorption of salt under the influence of desoxycorticosterone. In rats (329) and in dogs (308) polyuria could not be induced with desoxycorticosterone unless salt was given. The polyuria was preceded by an appreciable interval in which dogs retained water and gained weight (308). There was concomitant hemodilution (72, 308). The concentration of sodium in the serum rose (128, 308, 329), while its concentration in the urine fell (76, 308). Reduction of the urine volume by pituitrin did not cause the urinary concentration of chloride to rise as much as it does in true diabetes insipidus (308, 329). When dogs under the influence of desoxycorticosterone were deprived of water the polyuria subsided rapidly without the same degree of dehydration usually encountered in diabetes insipidus (308, 329). Cats with diabetes insipidus did not survive adrenalectomy as long as normal cats did (451, 452, 454). The negative sodium balance of adrenalectomy was not modified by diabetes insipidus; but serum sodium did not fall in cats with diabetes insipidus as it did in normal cats after removal of the suprarenal glands (454).

The thyroid gland. Because it accelerates metabolism the thyroid hormone increases water of oxidation and losses of water through the skin and lungs. The ratio of water lost by vaporization to heat production is abnormally large in hyperthyroidism (86, 237). In part, at least, this is referable to overactivity of the sweat glands, which may be diminished or prevented by atropine (237). In myxedema the proportion of heat eliminated by vaporization is unusually small (164).

In 1878 Ord (313) noticed the presence of a gelatinous fluid in the tissues of a patient who died of myxedema. Boothby and associates (43) estimated that this peculiar edema fluid, which disappears when thyroid preparations are given, contains about 2 per cent of protein. With its delivery sodium is lost, with only minimal quantities of potassium, indicating that the fluid and protein are deposited in the extracellular compartment (58). Elevated serum protein (98) and reduced blood volume (162, 397), which have been reported in myxedema, are logical consequences of the accumulation of protein in the interstitial spaces. In hyperthyroidism the circulating blood volume tends to be abnormally large (42, 162).

Claims that the thyroid hormone has a specific diuretic action (124) have not been substantiated. Mahoney and Sheehan (279) found that the polyuria produced in dogs by section of the hypophyseal stalk was inhibited by thyroidectomy and restored by thyroid therapy. After hypophysectomy diabetes in-

sipidus can be induced by extracts of either the thyroid or the anterior lobe of the pituitary gland (246, 433), suggesting that the anterior lobe acts through the thyrotropic principle (35, 246). Ingram and Fisher (229) could not diminish the diabetes insipidus of cats by thyroidectomy, and White (432) was unable to induce diuresis in hypophysectomized rats with either anterior lobe or combination of anterior lobe and thyroid. Swann and Johnson (391) diminished, but did not abolish, the polyuria of posthypophysectomized rats by thyroidectomy unless the animals were given salt. This suggests that removal of the thyroid, like removal of the anterior lobe of the hypophysis, alleviates the polyuria of diabetes insipidus only by diminishing the solutes requiring excretion. In man Cutler (41, 85) has reported some improvement of diabetes insipidus after thyroidectomy, Findlay (134) has reported none, while Jonáš (235) claims beneficial effects from thyrotropic hormone.

The sexual hormones. Thorn and his associates claim that a large number of the steroid sexual hormones promote retention of sodium and water, but the significance of some of the changes reported is doubtful (406). In the case of a castrate male there was simultaneous retention of nitrogen, potassium and phosphorus (400). Scharpey-Schafer and Schrire (357) were unable to alter the urine volume of subjects with estradiol. The incidence of menstrual edema in some women can be neither denied nor explained (16). There is suggestive evidence that progesterone may have an action resembling that of corticosterone (123, 401). The striking retention of fluid that accompanies tumefaction of the accessory sex organs of certain old world monkeys (254) is not relevant to humans. This fluid has the characteristics of extracellular fluid (71).

Pregnancy. Measurements of blood volume in pregnancy have, on the whole, yielded high values (99, 244, 346, 399), the chief exceptions being data of Schoenholz (350) by the carbon monoxide method. Thomson, Hersheimer et al. (399), using the dye T-1824, found that the plasma volume rose progressively from early pregnancy to reach the astonishing figure of 65 per cent above normal at the end of the ninth month. It then declined slowly to about 50 per cent above normal just before term and descended rapidly to normal in the first two weeks after delivery. The volume of circulating blood cells rose to a lesser degree. Others have reported smaller expansions. These increases are far out of proportion to the comparatively small rises of oxygen consumption and blood flow. Arterial blood pressure remains unchanged; venous blood pressure, except in the lower extremities, is not regularly elevated (56); disturbances of circulatory dynamics that might be anticipated from such a large addition to the circulation have not been discovered. One is forced to wonder whether, for technical reasons, the dye method does not give excessive values.

By means of thiocyanate Chesley (64) found that the extracellular fluid increased during normal pregnancy by an average of about 5 kgm. of which 1.5 kgm. could be accounted for by the uterus, membranes and fetus, an equal amount by expansion of the blood stream, leaving about 2 kgm. as general enlargement of interstitial fluid. About 1700 cc. of water were lost during delivery and about 2600 cc. in the first 6 days of the puerperium (65). Freyberg, Reekie

and Folsome (148) by metabolic measurements detected no increase of water during the last two months of pregnancy that could not be attributed to accessions of tissue. The problem is further confused by the extreme variability of the water retained by different individuals. Of 67 subjects studied by Chesley (64) over reasonably long intervals 6 gained from 0 to 50 ml. of water per week, 14 gained over 200. Of another group about 17 per cent lost, rather than gained, extracellular water during late pregnancy. Analyses of muscles of dogs (66) and of various tissues of guinea pigs (222) have revealed no evidences of abnormal quantities or distribution of water during pregnancy or after delivery.

THE EFFECTS OF DIET, WATER AND SALTS. *Starvation* is regularly attended by loss of a certain amount of water (33, 157, 442), part of which represents cellular water in which were dissolved protein and glycogen that are consumed (157, 442). Additional intracellular water and a larger quantity of extracellular water, with their salts, are also sacrificed. This Gamble, Ross and Tisdall (157) ascribed to the effects of starvation acidosis. This may play a part, especially in children who are peculiarly susceptible to ketosis; but in male adults Winkler, Danowski et al. (442) were unable to prevent the diuresis by 100 grams of carbohydrate daily, enough to minimize ketosis. Administration of water or salt solution does not prevent the initial dehydration (442). After 2 to 4 days an equilibrium is reached, after which water is lost in proportion to the tissue consumed (157).

Malnutrition (protein starvation). The origin of famine edema from protein deficiency was suggested (251, 292) before it was proved by experiment (149, 252). The edema results from reduction of serum albumin (50, 51, 423). It has been produced by low protein diets (149, 423). Serum albumin deficits have been observed in patients with a great variety of diseases and disorders accompanied by malnutrition (50, 51, 322). Transudation and depletion of serum albumin begin together at the very onset of protein starvation (122, 423), but edema at first is masked by loss of tissue, albumin deficiency by contraction of plasma volume. As much as 5 to 6 kgm. of fluid can accumulate in the interstitial spaces of an initially well developed adult male before edema is demonstrable by ordinary clinical methods (320). If an individual has some other disorder conducive to edema the effect of hypoalbuminemia is exaggerated. In cardiac disease edema will appear or persist with a lesser degree of decompensation in malnourished patients with slight serum albumin deficits (316). The appearance of ascites and dependent edema in cirrhosis of the liver usually signifies that there is an associated hypoalbuminemia (57, 322).

Excesses of water. After moderately large volumes of water (1 to 1.5 liters) diuresis does not begin until absorption is far advanced and long after there has been detectable reduction of the sodium and chloride concentrations in the serum; it reaches its peak only after absorption is completed (360, 361). The diuretic response, presumably effected by inhibition of posterior pituitary activity, is probably elicited by expansion of blood volume. The initial fall of plasma electrolytes does not signify dilution, but the passage of salt into the alimentary canal. It may even coincide with contraction of the extracellular

fluid because the loss of salt will cause cells to take up water. The excretion of small or moderate increments of water is accomplished by reduction of reabsorption alone; large amounts increase glomerular filtration, probably because they swell the blood volume and raise glomerular capillary pressure (354, 355). Forcible administration of extremely large quantities of water provokes toxic symptoms, culminating in convulsions, collapse and death. These are referable not to the expansion of body fluids, but to the reduction of electrolyte osmotic pressure and consequent swelling of tissue cells. They can be allayed by hypertonic saline in spite of the fact that this further swells the body fluids (190 344). On the other hand, concentrated urea solutions are of no benefit because the urea diffuses freely across the cell membranes and therefore does not correct the maldistribution of water (190).

Water deprivation. When animals are deprived of water insensible perspiration diminishes as dehydration becomes extreme (37, 91, 121, 442). Urine volume diminishes rapidly, presumably under the influence of the posterior pituitary hormone, until only enough is voided to eliminate in less than maximal concentration the solutes requiring excretion. It is greatly influenced by the rate of protein metabolism (442). Conservation of water is accomplished by augmented reabsorption until the urine volume has reached a minimum and its concentration has become maximal, when glomerular filtration falls, probably because the reduction of blood volume and the inspissation of the blood lower the glomerular capillary pressure and raise the colloid osmotic pressure of the plasma (353). The blood urea rises. As dehydration advances water is lost out of proportion to sodium and chloride which are preferentially absorbed. Besides water is lost in the insensible perspiration without salt, leaving only a fraction of the water to carry into the urine the salt which it held in the body. The concentrations of sodium and chloride in the extracellular fluids rise and, by increasing the osmotic pressure, draw water from the cells. The cells also yield a certain amount of potassium which permits the escape of further cellular water (37, 119, 120). Moderate dehydration is compatible with physical activity although the latter, by accelerating extrarenal dissipation of water, aggravates the dehydration. Physical efficiency becomes impaired as dehydration progresses and loss of 10 per cent of the body water is totally disabling to men (442). In dogs death occurs, apparently from respiratory failure, when 25 per cent of the body water has been lost (91). Because it can concentrate urine more efficiently than man the dog can subsist on a high protein diet with less water. The dog can subsist upon a diet of fish with no more water than can be obtained from the fish; man on a similar diet is forced to expend some of his own body water for the excretion of the nitrogen derived from the fish (442).

Sodium deficiency. It is impossible by mere dietary restriction of salt seriously to deplete an animal of sodium, because the urinary output of sodium and chloride falls so rapidly. Greenberg and Cuthbertson (175) found that rats could maintain chloride equilibrium, and even store chloride, on as little as one milligram per day. The effect of withdrawing sodium by excessive sweating, vomiting, diarrhea, etc., without replacing it, depends upon the amounts of water

simultaneously lost and the amounts taken to replace these losses. When Darrow et al. (95, 348) removed salt without water from the bodies of dogs by injecting glucose solutions intraperitoneally and subsequently withdrawing the fluid, the concentrations of sodium and chloride in the extracellular fluids fell and the volume of these fluids diminished because the cells swelled. In such acute experiments there is no immediate effort of the kidneys to restore the composition of the body fluids. A state resembling shock (348) ensues with almost complete anuria. This may arise from reduction of the blood volume (300) associated with circulatory failure. The animals evince little thirst (95, 165). If they are allowed access to water and are given salt-poor diets the sodium and chloride concentrations and the volume of the extracellular fluids remain low. The kidneys will not preserve electrolyte osmotic pressure to the total neglect of the volume of the extracellular fluid.

Loss of both water and salt in hypotonic solution, as in sweat, is comparable in its immediate effect to a primary loss of salt. Sweating greatly accelerates the dehydration of water deprivation, including the increases of sodium and chloride in the serum. If the losses of salt have been great, as they may be when heavy work is done at high temperatures, replacement of the lost fluid by drinking water may precipitate muscular and abdominal cramps (188, 306) and other symptoms of "water intoxication". Although the concentrations of sodium and chloride in the extracellular fluids are elevated, the total amounts of these ions in the body are low. Replacement of the fluid by water alone, therefore, produces hypotonicity. During actual work water may be taken to supply sweat. In the intervals between activity, when sweating has ceased and water is taken to reconstitute the body fluids, salt is required (103, 395). This is less necessary after acclimatization is complete. Salt taken during activity merely aggravates thirst and dehydration. Ingestion of salt with insufficient water is highly undesirable because it drains more water into the urine when the kidneys are making every effort to conserve water. Ladell (255) and others claim that men marooned at sea may benefit by supplementing scanty supplies of fresh water with sea water. The evidence is not convincing that these supplements actually spare any body water (442). The concentration of salts in sea water approaches or exceeds the concentrating powers of the human kidney. Drinking sea water alone is, therefore, disastrous. Sea water enemas are equally dangerous (48, 143). Since the osmotic pressure of the contents of the gut can not be raised above the osmotic pressure of the body fluids, such enemas withdraw water into the gut and further raise the concentrations of sodium and chloride in the body.

Because gastrointestinal and digestive secretions are isotonic their loss through vomiting, diarrhea and fistulae should deplete sodium and water in equivalent proportions. Actually, since water continues to be dissipated in the insensible perspiration, if no food or fluids are given, more salt than water would be wasted. If water is ingested a certain amount is absorbed, the remainder discharged in the vomitus, diarrhea or fistulous matter, carries with it salt that has been secreted into the water to make it isotonic. These disorders, consequently,

usually produce a state of primary salt depletion (150, 156, 204). Since the volume of vomitus, especially in obstruction or irritation of the gastrointestinal tract, usually exceeds the volume of fluid ingested, the salt depletion is associated with a large deficit of water. By prohibition of oral food and fluids the vomiting and diarrhea of mercury poisoning may be abolished (323), the vomiting of pyloric obstruction can be greatly reduced (142, 318). If decompression and lavage of the obstructed gut are practised (1, 2, 420) lavage should be performed, not with water, but with normal saline, to reduce secretory activity of the alimentary canal and the digestive glands to a minimum (83, 319). The discharge of fluid from high intestinal fistulae may be greatly diminished by giving only isotonic solutions of saline and glucose (320).

Sodium excess. If the ratio of sodium salts to water is greater in the ingesta than in the extracellular fluid, the volume of the latter expands at the expense of the cells (95, 444). Diuresis ensues, its intensity depending upon the volume and salt concentration of the fluid given (196, 442). The diuresis is due chiefly to the limitation imposed upon tubular reabsorption of water by the osmotic effect of the salt. Glomerular filtration is increased more by a given dose of hypertonic saline than by an equal amount of water (355) perhaps because the former, by drawing water from the cells, expands the extracellular fluid and the volume of the blood further.

Acidifying measures. If a strong acid which can not be utilized, for example a mineral acid, is given, it forms sodium salts in the body, displacing bicarbonate. The pH of the body fluids falls and, for reasons discussed above, the cells swell. Since the kidneys can not eliminate the acid without some of the sodium with which it is combined, they respond, as they do to primary sodium deficiency, by sacrificing some water in the interests of the electrolyte osmotic pressure of the body fluids (14, 151, 158). Ammonium and calcium chloride have acidifying effects similar to that of hydrochloric acid. The ammonia of ammonium chloride is converted to urea, leaving the chloride ion to form hydrochloric acid. Calcium chloride is acidifying only when given by mouth, when a large part of the calcium is excreted in the feces, while the chloride is absorbed (14, 151, 158).

Alkalinizing measures. Administration of the hydroxide, carbonate or bicarbonate of sodium or sodium salts of organic acids that can be oxidized increases the sodium, bicarbonate and pH of the extracellular fluids. The rise of pH causes the cells to shrink. If sufficient water is available the excess of bicarbonate is eliminated by inhibition of its reabsorption in the tubules. Sodium excess has, therefore, little diuretic effect; in pathologic states it may even conduce to edema (150, 156, 204). Spontaneous sodium excess has not been reported. Far commoner is replacement of chloride by bicarbonate, the condition encountered in patients who have vomited acid gastric contents (150, 156, 204). In such states there is invariably dehydration and sodium deficiency. The sodium released by hydrochloric acid forms bicarbonate which is excreted by the kidneys to restore the normal anion pattern. This leads to a sodium deficit that is met by sacrifice of water (150, 156).

Salts other than sodium bicarbonate and chloride, which distort the chemical

pattern of the body fluids are eliminated by the kidney with as much water as is required to permit their excretion in less than maximum concentration. Because of the efficiency with which sulfate is excreted and its comparative physiological inactivity, its salts have a strong diuretic effect if they gain access to the body (38, 151, 351). They are, however, so slightly absorbed that, when given by mouth, they induce diarrhea; for diuretic purposes they must be given parenterally. As a diuretic sodium sulfate is to be preferred (351); the magnesium salts, because of the toxic action of the magnesium ion, can not be given in large enough quantities (373, 447). The phosphate ion has other actions that contraindicate its general use as a diuretic. The body seems peculiarly tolerant to nitrate, accepting it as a substitute for chloride (223). Keith (245) has recommended ammonium nitrate as an effective acidifying diuretic. The chloride and organic salts of potassium are more diuretic than equivalent amounts of corresponding sodium salts, but can not be given in large enough quantities to have an important effect on urine volume.

DIURETIC DRUGS AND CHEMICALS. *Urea* is perhaps the blandest of all diuretics. Diffusing freely through all the fluids of the body, without appreciable physiological activity, it does not even alter the distribution of water. It can, therefore, be given in large quantities (30, 82, 277). It acts by limiting the reabsorption of water in the renal tubules.

Sugars, when taken by mouth, have no significant effect on water metabolism except as they prevent ketosis, reduce protein catabolism and provide water of oxidation. Isotonic glucose solution injected intravenously is equivalent from the standpoint of water metabolism to the injection of an equal amount of water because the glucose diffuses rapidly throughout the body and is consumed by the cells. If the same solution is injected into a serous cavity or subcutaneously the water is absorbed more slowly than it would be from isotonic saline since salt diffuses into the glucose solution more rapidly than glucose diffuses out. Initially such an injection may abstract water from the body at large (95) because, until equilibrium is established between the two media, the osmotic pressure is higher in the injected pool than in the rest of the body. If hypertonic glucose solution is injected rapidly into a vein it draws fluid into the blood stream until the sugar has had time to diffuse out of the circulation. This may elicit transient diuresis; but glucose exerts no important diuretic action unless it gives rise to glycosuria, because it is injected more rapidly than it can be utilized, because its utilization is impaired, or because its reabsorption in the renal tubules is inhibited by phlorizin. Other monosaccharides have a similar effect if they are absorbed or injected more rapidly than they are consumed. Disaccharides, with the exception of maltose, are not utilized if they enter the blood stream, but are rapidly and completely eliminated in the urine. Because of its large molecular weight a given amount of disaccharide should command only half as much water as an equal weight of glucose. This is partly offset by the fact that disaccharides escape reabsorption almost or quite completely and are excluded from cells from which they extract water by their osmotic effect.

The purine and mercurial diuretics act directly upon the kidney (24, 34, 174).

There is some evidence that they inhibit primarily reabsorption of sodium chloride (270, 343), in which case the diuresis of water may be a secondary reaction. In keeping with this both the purine drugs (302) and the mercurials (270) in diabetes insipidus enable the kidneys to concentrate chloride in the urine, and therefore have an antidiuretic rather than a diuretic action in this condition. According to Unna and Walterskirchen (409) pituitrin augments the diuretic effect of these drugs.

POSTURE AND EXERCISE. Stationary maintenance of the erect posture or a position in which the legs are immovably dependent leads to hemoconcentration with swelling of the lower extremities (398) because, under the influence of gravity venous stasis becomes great enough to cause transudation while lymphatic drainage is retarded (105). This edema can be prevented or eliminated by exercise of the lower extremities because muscular contraction facilitates the flow of blood and lymph (293).

Severe muscular exercise, by accelerating energy expenditure, increases water of oxidation and vaporization from lungs and skin (192), as well as blood flow. Hemoconcentration, which has been reported (23, 342), is usually attributed to contraction of the circulating blood volume. Ebert and Stead (108) found that the blue dye T-1824 could not be used to measure plasma volume in exercise because the inherent optical properties of serum are altered by exercise.

During exercise muscles lose potassium (125, 180, 303), according to Miller and Darrow (303), with a proportional quantity of organic solid, probably glycogen.

Severe exercise diminishes the rate of formation of urine (79, 111, 212, 345). This is not due only to the diversion of water to other excretory channels nor to hemoconcentration, since it is observed even when exercising subjects drink water (111, 212). Rydin and Verney (345) believe it is referable to action of the antidiuretic hormone of the pituitary. If, however, glomerular filtration is diminished, as Covian and Rehberg (79) claim, there may be in addition a disturbance of renal circulation. The excretion of chloride falls (111, 207) and the urea clearance diminishes more than the creatinine clearance does (79). Both the intensity and the duration of the oliguria vary directly with the severity of the exercise (79) and the oliguria persists for some time after exercise has ceased (207, 212).

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